ACTA MEDICA SCANDINAVICA

SUPPLEMENTUM 162

DISCRETE GAMMAGLOBULIN (M-)COMPONENTS IN SERUM

CLINICAL STUDY OF 150 SUBJECTS WITHOUT MYELOMATOSIS

by

JAN HALLÉN

MALMÖ 1966

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has been published since 1919 as a continuation of Nordiskt Medicinskt Arkiv, founded in 1869 by Axel Key The first volume of Acta Medica Scandinavica is therefore numbered LII (52)

The chief editors have been. Axel Key 1869–1900, C G Santesson 1901–1915, I Holmgren 1916–1957 and Birger Strandell 1958 to date

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FROM THE DEPARTMENT OF INTERNAL MEDICINE UNIVERSITY OF LUND
MALMÖ GENERAL HOSPITAL, MALMÖ SWEDEN
HEAD PROFESSOR JAN WALDENSTRÖM

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Translated by Mr L James Brown

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CONTENTS

KEY TO TERMS AND SYMBOLS USED

The term component always refers to M component (discrete component) The expressions γG component γA component and γM component are thus to be understood as M components of types γG γA and γM

The term gamma fraction is to be understood as the paper electrophoretic gammaglobulin fraction excluding M-component if any of the same mobility

The term normal range is usually that used at the department of clinical chemistry in Malmo (mean ± 2 S D)

Certain patients had been treated with melphalan (Alkeran® L bis-chloroethylamino phenylalanine)

If a case report is given in the appendix the number of the case in the text and tables is underlined

The cases of myelomatosis are characterised by the letter m after the case number and Roman numbers are used to designate cases with γM components

INTRODUCTION

The electrophoretic pattern of serum occasionally shows an extra fraction in the range of the immunoglobulins. In free boundary electrophoresis such a fraction is seen as a sharp peak. Such a peak is also seen on graphic demonstration of the electrophoretic pattern of the paper electrophoretic strip at the site corresponding to a narrow band. Such a band is called an M component (Riva 1957), a discrete component or a pathological protein (WHO meeting on nomenclature of human immunoglobulins 1964).

In the immunoelectrophoretic pattern the M component is usually seen as a bow of great density, intense convexity towards the antibody reservoir, and short extension in the electrophoretic migration direction, all features indicating a very localized and important excess of some antigen (Heremans & Heremans 1961). An M component can be classified immunochemically and assigned to one of the groups γG, γA, γM (see Heremans 1960) or γD (Rowe & Fahey 1965). An M component can also consist of a Bence Jones (light chain) protein (Moore et al. 1943) or a fragment similar to the heavy chain (Franklin et al. 1963) in the immunoglobulin molecule (Porter 1962).

M components were first found in myelomatosis. Since then such components have been demonstrated in sera not only from patients with macroglobulinaemia Waldenström but also from patients with other diseases and from apparently healthy persons.

The diagnostic as well as the prognostic significance of a serum M component in the 2 last mentioned groups is still obscure. This problem will be treated in the present investigation, which is concerned with 150 subjects with discrete serum components but in whom the diagnosis of myelomatosis must be regarded as excluded, unlikely or doubtful, and with 92 cases of myelomatosis from one community.

matosis (Osserman & Takatsuki 1965)) the bone marrow contained 65—25 % plasma cells Some of the subjects were clinically well but strikingly many of the others had pulmonary infections

Immunoelectrophoretic analysis of 11 sera with M components from patients without signs of myelomatosis ("dysprotéinémies atypique") was performed by Creyssel et al (1959) In 2 cases the M components were of type γM and in one of type γA The concentration of the M components ranged between 0 5 and 4 0 g/100 ml Five cases were found to have more than 20 % lymphoid cells in the bone marrow smear 4 cases had 7—9 % plasma cells and one case with liver cancer had 18 % atypical plasma cells (Morel et al 1959) Skeletal x ray revealed no evidence of myelomatosis In 4 cases the diagnosis was reticulosis or reticulo lymphosis and in one lympho monocytic leukaemia

Hammack et al (1959) described 4 men with serum M components Ultracentrifugation showed no abnormal macro globulin fraction The bone marrow and skeletal x ray produced no support for the diagnosis of myelomatosis and Bence Jones test was negative The observation period varied between 9 months and 3½ years In 3 of the cases there was an increased tendency to infection e g pneumococcal sepsis 5 times in one and pneumonia 7 times in another One patient later developed plasma cell leukaemia (Hammack et al 1964)

Ogryzlo et al (1959) reported 10 cases with M components In one case the findings were compatible with a diagnosis of macroglobulinaemia Waldenström Cryoglobulinaemia and an increased number of plasma cells in the bone marrow were noted in one man Otherwise there was no bone marrow plasmocytosis and x ray disclosed no osteolytic lesions The diagnoses were e g lymphosarcoma lymphoma, sclero dermia, rheumatoid arthritis

Owen et al (1959) reported 9 cases with M components found on paper electrophoresis Ultracentrifugation was done in 8 of the cases In 3 the M component was a macroglobulin (2 0—3 7 g/100 ml) The concentration of the gammaglobulin fraction in the other sera ranged between 1 5 and 7 1 g/100 ml The latter value was seen in a patient with "lymphosarcoma or some related condition" (according to histological examination of a biopsy specimen from the iliac crest) and punched out lesions of the skull One patient had anaemia, a cystic rarefaction in the left ileum and a gammaglobulin concentration of 5 3 g/100 ml The bone marrow contained 9 % plasma cells in one of the cases with macroglobulinaemia No cytological signs of myelomatosis were found in the other cases Skeletal x ray showed osteolytic lesions also in a patient with thyroid cancer Besides the above mentioned conditions there were e g peptic ulcer, recurrent pneumonia haemolytic anaemia Three patients had been followed up for 2—3 years

Nine cases with serum M components were examined by Kohn (1960) The diagnoses were reticulum cell sarcoma or reticulosis in 3 cases haemolytic anaemia in 2 cirrhosis in 2 cerebral thrombosis in one, and one subject was a pregnant woman

Kyle et al (1960) reported 15 subjects with M components In 8 of them there was more or less certain macroglobulinaemia In the remaining 7 the diagnoses given were e g amyloidosis (primary and secondary) lymphoma

M components were found by Schobel & Wewalka (1961) on paper electrophoresis of serum from 6 patients without evidence of myelomatosis Ultracentrifugation excluded macroglobulinaemia There was no Bence Jones protein The concentrations of the

CHAPTER I
HISTORICAL

The first report suggesting an abnormal protein synthesis in myelomatosis was published by Bence Jones in 1848. Abnormal properties of sera from patients with myelomatosis have since been described as an anticomplementary activity by Citron (1921), as cryoglobulinaemia by Wintrobe & Buell (1933) and von Bonsdorff et al (1938), as a high peak in the ultracentrifuge diagram by McFarlane (1935) and in the electrophoretic pattern as an M component (Longsworth et al 1939, Kekwick 1940). Such components have since been *demonstrated in serum and/or urine in* 99 % of a large series of myelomatosis (Osserman & Takatsuki 1963).

In 1944 and 1948 Waldenström described the constellation of clinical, cytological and protein chemical findings now known as macroglobulinaemia Waldenström and then described discrete components in a condition other than myelomatosis.

Another disease ("heavy chain disease") with signs of an abnormal activity of the immunoglobulin producing system, *inter alia* in the form of M components in serum and urine has been described by Franklin et al (1964) and Osserman & Takatsuki (1964).

Waldenström (1952) pointed out—also as the first—that M components that were not macroglobulins sometimes occurred in persons without clinical cytological or roentgenological signs of myelomatosis. *The condition was described* as "essential" hyperglobulinaemia and was demonstrated in 2 cases with an increase of the E S R (69 and 93 mm/1 hr) known for 5 and 6 years respectively. One of the patients complained of dizziness and had mild hypertension, the other had slight cardiac incompensation.

A longer follow-up of 2 similar cases (20 respectively 7 years) based on the E S R increase was described by Olhagen & Liljestrand (1955). Both subjects felt well, the bone marrow appeared normal and roentgen examination showed no osteolytic lesions.

Smith (1957) reported 13 subjects with M-components in the serum without signs of myelomatosis. Two exhibited the clinical picture of macroglobulinaemia Waldenström. The M component was usually the only serum abnormality, but in some cases cryoglobulinaemia was noted, and sometimes the concentration of the paper electrophoretic gamma globulin fraction was decreased. A slightly increased number of plasma cells in the bone marrow was common. In some cases followed up for 4 years or more no change suggesting myelomatosis occurred in the clinical picture or the appearance of the bone marrow. Some subjects were "clinically well". Of the others, many had chronic infections of the lungs.

Osserman (1958) reported 21 subjects studied for a few months to more than 3 years after the M component had been demonstrated by paper electrophoresis. The concentration of the M components had remained constant or increased. In 2 cases macroglobulin components were found on ultracentrifugation. Neither bone marrow smears nor skeletal x ray produced any evidence of myelomatosis, but in 3 cases (metastasising breast cancer, liver cirrhosis, cholecystitis but later unequivocal myelo

increase of the total protein or any sign of myelomatosis in the bone marrow smear The duration of follow up was not given The diagnoses were *inter alia* bronchial cancer in 2, disseminated lupus in 2 and cholangitis in 2 cases

In 10 cases with serum M components Hallén (1964) found nothing suggesting myelomato is or macroglobulinaemia Waldenström Cancer was diagno ed in 4 cases (1 γA 3 γM) and lymphatic leukaemia in one (γG) Five cases (4 γG 1 γA) were conceived as "es en tial

Huhnstock et al (1964) de cribed 16 cases of ' symptomarmen Paraprotein ämien The immunological types were γG in 11 cases γA in 4 γM in one and γμ in one In 6 cases the total protein was more than 8 0 g/100 ml In 12 an M component was demonstrated by immunoelectrophore is of concentrated urine Skeletal x ray, which was repeat edly done during the follow up (6 months to 9 years mean 3 3 years) did not reveal any lesions The bone marrow in 10 of the cases showed changes varying from a slight diffuse increase of typical plasma cells to single pathologic cell proliferations In some cases the diagnosis was given (*e g* diabetes osteoarthritis *hepatomegaly*)

Five patients with 'idiopatische Para proteinämie were described by Radl & Masopust (1964) one had a γM component (2 3 g/100 ml) the others γG components (1 0—1 5 g/100 ml) Only one of the latter had a light chain component in the urine Marrow punc ture of the sternum, of the spinous process of a vertebra and of the iliac crest haematologic and roentgenologic studies revealed no evidence of myelo matosis The follow up varied between 2 and 7 years Three of the patients had cardiova cular di eases one had diabetes and one was an alcoholic with liver damage This material was later supple mented by 3 patients with γG compo nents of about 1 0 g/100 ml and without cytologic or roentgenologic evidence of myelomatosis Cancer was diagnosed in 2 of them and suspected in the third (Radl et al 1965)

Riva (1964) noted ' idiopatische und Begleitparaproteinämie in 33 out of 250 patients with M components The com ponents were of type γG in 20, γA in 10 and γM in 3 Bone marrow puncture had been done at several sites and on various occasions but neither the bone marrow nor the roentgen appearance of the skeleton suggested myelomatosis The follow up varied between 2 and 12 years and in 8 cases necropsy was done The diagnoses were *e g* cancer (10 cases) tuberculosis sarcoidosis, liver cirrhosis diabetes arteriosclerosis

Videbæk & Drivsholm (1964) reported 10 cases with M components The com ponent was of type γG in 8 γA in one and γM in one The concentration of the components ranged between 0 7 and 5 7 g/100 ml Neither bone marrow smears nor skeletal x ray suggested myelomatosis The diagno es mentioned were primary amyloidosis (2 cases) polycythaemia vera (2) disseminated lupus liver cirrhosis and chronic lymphatic leukaemia

Waldenström (1964 a) reported a number of subjects with M components (γG γA) but without evidence of myelomatosis The subjects had been followed up for more than 2½ years or if they had died earlier necropsy had been performed (Several of these cases are included in Chapter III of this presen tation where they have the same iden tification numbers—see Table XIII Some of them have been a signed to the myelomatosis group because of observa tions made during the later course or the u e of different diagnostic criteria and some cases conceived by Waldenström as examples of myelomatosis were for the ame reasons not cla sified as such in the pre ent inve tigation) The mate

M components can be estimated at 1 0—2 0 g/100 ml The bone marrow did not contain more than 6 % plasma cells None of the patients was anaemic Skeletal x ray showed osteoporosis in 2 cases The follow up was 8 months to 3½ years The patients had sought medical advice for symptoms of *e g* pneumonia hypertension gastritis

Waldenstrom (1961) published a clinical analysis of a large series of subjects with M components Of 276 subjects 18 (3 with discrete component of type γM) followed up for a long time developed no symptoms or signs of myelomatosis or macroglobulinaemia Waldenström These 18 were classified as essential hyperglobulinaemia

Similar cases were observed by Hurlimann & Martin (1962) In 2 cases there were γM and in 3 γG components An increased number of plasma cells was found in the bone marrow from one patient with liver cirrhosis X ray revealed no osteolytic lesions The follow up was 1—3 years in the 3 cases with M components of type γG The diagnoses were cirrhosis (2 cases) suspected pulmonary amyloidosis malabsorption syndrome of unknown origin and cancer of the urinary bladder

Refvem & Bjornstad (1962) described a man who had a serum M component and who died from pulmonary cancer The globulin fraction varied between 3 9 and 5 2 g/100 ml No Bence Jones protein could be demonstrated The number of bone marrow plasma cells was increased but necropsy showed no evidence of myelomatosis They also reported an alcoholic with repeated pneumonia and obscure rectal bleeding a γG component of 3 0 g/100 ml a patchy increase of the plasma cells in the bone marrow but no x ray evidence of osteolytic lesions The condition had remained unchanged for 3 years

The youngest known subject (Stoop *et al* 1962) with an M component is a 3 month old girl Some of her siblings had died in infancy from persistent infections and she herself died at 7 months in fever with otitis pneumonia and diarrhoea In addition to an immunoglobulin deficiency "paraproteins" deforming the γG line were noted The spleen thymus and lymph node showed a severe loss of lymphocytes but the presence of numerous plasma cell The number of plasma cells in the bone marrow was not increased

The term "rudimentare Paraproteinamie" was used by Marki & Wuhrmann (1963) to designate 6 cases with M components of a concentration of about 1 0 g/100 ml This group was supplemented in 1965 by a further 8 cases Eleven had γG and 3 had γA components In several of the case the concentration of the gamma fraction was decreased Bence Jones protein was demonstrated in the urine in only one patient The number of plasma cells in the bone marrow was 8 % in one case 5% in 5 and lower in the others Skeletal x ray showed osteoporosis in 9 cases including 2 where x ray suggested osteolytic lesions in the skull The duration of follow up was not mentioned but it cannot have exceeded 3 years The diagnoses were *e g* peptic ulcer pancreatitis alcoholic polyneuritis and fatty liver cardiosclerosis

Of 400 subjects with M components Osserman & Takatsuki (1963) classified 71 as monoclonal gammopathy of unknown origin In spite of prolonged observation for periods of up to 9 years they had not developed the clinical picture of myelomatosis or macroglobulinaemia Waldenstrom Neoplasms other than lymphoma had been diagnosed in 31 patients The immunological type was γG in 60 γA in 8 and γM in 3

Twelve cases with γG components seen on paper electrophoresis were published by Scheurlen (1963) In none of the case was there any appreciable

increase of the total protein or any sign of myelomatosis in the bone marrow smear The duration of follow up was not given The diagnoses were *inter alia* bronchial cancer in 2 disseminated lupus in 2 and cholangitis in 2 cases

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nal lent support to the assumption that a high and/or increasing M component concentration argues for a diagnosis of myelomatosis That the increase in the M component concentration in myelomatosis may sometimes be initially slow was exemplified by a few cases Severe anaemia and hypoalbuminaemia of obscure origin were regarded as arguing for myelomatosis In most cases there was little (3—5%) or no plasmocytosis of the bone marrow

At the Xth Congress of the International Society of Haematology 3 papers were read on cases of relevant interest Danon & Seligmann (1964) presented 30 cases with γG- or γA-component but without clinical, roentgenologic or haematologic signs of myelomatosis The components were usually of moderate concentration A low concentration of the normal immunoglobulins was less common than in myelomatosis Sometimes Bence Jones proteinuria was demonstrated The diagnosis was cancer in 6 cases, lymphatic leukaemia in 4, cirrhosis in 6, collagenosis in 5, amyloidosis in one and myelofibrosis, polyneuritis and spondylosis in others Several of the patients were young and the youngest was a 5 month old boy with Aldrich's syndrome

In an investigation of 250 persons above 70 years Ime et al (1964) found some with M components in the serum, but without corroborating evidence of myelomatosis or macroglobulinaemia Waldenstrom In about 10 the lymphatic or plasmacellular system seemed to be affected, but in 7 clinical and haematologic investigation was unrewarding

Imhof et al (1964) had sometimes diagnosed "silent" cirrhosis at liver biopsy in patients with M components which had not myelomatosis or macroglobulinaemia Waldenstrom

Bachmann (1965) analysed 554 sera with M components and collected clinical data justifying the following classification clinically manifest or assumed myelomatosis in 244 cases, malignant lymphoma including macroglobulinaemia Waldenstrom in 57, undifferentiated neoplasia of the reticular tissue in 7, cancer in 49 and other conditions in 197 The frequency of myelomatosis and malignant lymphoma increased with the concentration of the M-components The majority of the cases in the present investigation were included in Bachmann's material

In 1965 Brittinger & König reported a series of cases with serum M components found on examination of 5,500 patients In 8 cases the diagnosis of myelomatosis or macroglobulinaemia Waldenström could not be proved, but they were followed up for only a short time The concentration of the M components ranged between 0 3 and 1 7 g/100 ml and all were of type γG A light-chain component was suspected in the urine in 2 cases The number of plasma cells was between 6 and 9 %, but in 2 cases it was 15 % In these 2 cases myelomatosis was suspected also because of co existing osteoporosis

Some authors have described cases with M components which they thought to be related to one disease or another mostly leukaemia, lymphosarcoma or reticulum cell sarcoma (Chapter V)

Christenson & Dacie (1957) Christenson et al (1957) and Fudenberg & Kunkel (1957) reported cases with haemolytic anaemia and produced evidence strongly suggesting that the components consisted of cold agglutinins

In 4 of 6 cases with pyoderma ulcerosa serpiginosa Röckl et al (1964) found M components of type γA in a concentration of about 1 0 g/100 ml In 3 cases examined the bone marrow contained an increased number of plasma cells, which was also observed in one of 2 cases without M component The increase in the number of plasma cells was moderate except in one case in

which myelomatosis must be strongly suspected

Some authors (Wieme 1959, Engle et al 1961 Oriol Bosch & Tamm 1961 Brackenridge & Csillag 1962 Lohmann 1964) have used stabilizing media other than paper Although their series contain cases with M components but without symptoms or signs of myelomatosis or macroglobulinaemia Waldenström they will not be commented upon here

CHAPTER II

MATERIAL AND METHODS

MATERIAL

The primary material consisted of all cases of *myelomatosis or local plasmocytoma in Malmo* and diagnosed at Malmo general hospital the only hospital in the town between January 1 1950 and June 30 1964 as well as all cases from Malmo with a serum M component discovered by paper electrophoresis between the time of *introduction of the method (end of 1953)* and June 30 1965

CLASSIFICATION AND SELECTION

Cases satisfying the following criteria were accepted as myelomatosis

1) Roentgenographic osteolytic lesions (not only osteoporosis or vertebral compression) for which the only explanation found was an increased proliferation of plasma cells seen at necropsy or histologic examination of a biopsy specimen Forty six cases satisfied these criteria

2) Gross focal post mortem skeletal changes corresponding to increased plasma cell prolifera tion Twenty three cases satisfied this criterion In 4 further cases (21m 61m 89m 91m) examined post mortem but in which no osteo lytic lesions had been demonstrated roentgeno logically or on gross examination at necropsy the increase of the plasma cells in the bone marrow was so pronounced that the pathologist made a diagnosis of myelomatosis without hesitation In these cases which were also assigned to the group of myelomatosis no signs of any other disease possibly responsible for the plasmocytosis were found (p 26)

3) Roentgenographic osteolytic lesions for which no explanation could be found except an increased number of plasma cells in bone marrow smears (more than 3 % Gormsen 1942 p 38) This criterion was filled by 16 patients 14 of whom were still living Case 59m and the following 2 cases were also accepted in spite of the fact that no osteolytic lesions were seen

Case 62m After a period of chest pain para plegia occurred X ray showed vertebral com pressions The bone marrow contained 9 % plasma cells and the serum an M component (0 7 g/100 ml) The patient was treated with melphalan only and improved When last seen 2 *years later she* was up and about

Case 82m A period of chest pain was followed by paraplegia X ray showed pronounced osteoporosis and vertebral compressions No serum M component hypogammaglobulinaemia (0 3 g/100 ml) Bence Jones test negative and 66 % *plasma cells in the bone* marrow The patient died 3 months later Necropsy was not done

Three patients (J J b 1877 J B b 1896 H N b 1890) were not accepted In these cases paraplegia was caused by local plasmocytomas *and the disease* was diagno ed incidentally at post mortem Bone marrow puncture had not been done and the serum had been studied electrophoretically in only 2 of them and in neither had it shown the presence of an M component or hypogammaglobulinaemia

The remaining cases were divided into 3 group

I All cases with an M component of type γG or γA and 4 cases in which immunoelectro phoresis had not been done or had not revealed the type of the component

II All cases with an M component of type γM

III All cases with *leukaemia* lymphosarcoma or reticulum cell sarcoma

Of groups I and II all cases were *excluded* that had not been checked by electrophoresis bone marrow puncture and skeletal x ray one year or more after the discovery of the M component *unless necropsy had been performed and* as far as cases belonging to group I are concerned marrow from at least one bone had then been examined histologically Four patients could not be included because they would not or could not co operate at the after examination 39 becau e *the M component had been discovered too* recently and 24 because necropsy had not been performed or had been incomplete

FOLLOW UP

When an M component had been discovered supplementary examinations (especially sternal puncture and skeletal x ray) were performed and then usually while the patient was still in hospital Those patients who no longer required treatment were requested to attend the outpatient depart ment for follow up The patients were followed up by Prof Waldenstrom until *1961 and then by the* author At after examination in *1963* physical examination was supplemented *by routine* haematologic examination paper electrophoresis of the serum and skeletal x ray and in cases in which the bone marrow had not previously been examined also by sternal puncture

All subjects available *were after* examined in spring *1965 The* examination included physical

examination sternal puncture and puncture of the spinous proce s of a lumbar vertebra It al o included x ray of the skull *ribs thoracic and* lumbar spine pelvis and proximal parts of the femora Determinations were also made of the Hb R B C. W B C. thrombocytes reticulocytes serum creatinine and calcium as well as of any anticomplementary activity of the serum Paper electrophoresis of the serum was performed and in ca es with proteinuria also of the urine Of 62 living subjects in group I 53 co-operated Two (Nos 2 and 6) in whom myelomatosis was su pected and who were receiving or had received melphalan were re examined in 1965 by the physician in charge of them In 3 cases (Nos 27 42 55) in which the M components had previous ly disappeared the examination was limited to paper and agar gel electrophore is Four subjects could not be included in the after examination one of them (No 28) had moved from Malmo but his M component had been demonstrable for only a short time one (No 20) had died from *myocardial infarction but necropsy had not been* performed one (No 14) could not attend the outpatient department becau e of severe arthro is of the knee joint one (No 8) was suffering from depres ion and would not consent to more than phy ical examination and blood sampling at home All 4 subjects had been after-examined completely more than one year after the discovery of the M component —Of 13 living ubjects in group II Nos IV and XIII did not co-operate Both had moved from Malmo but the M-components had disappeared soon after detection Paper electrophoresis of the serum was however performed in case IV

METHODS

Protein studies were done according to methods de cribed by Bachmann & Laurell (1963) and discussed by Bachmann (1966 a) The total protein was measured with a biuret method paper electrophoresis was run in a calcium lactate containing barbitone buffer For immuno chemical classification immunoelectrophoresis was performed with the use of specific rabbit anti era against γG γA γM and human immuno globulins as well as horse antihuman antiserum When nece sary immunoelectrophoresis was done after cysteine treatment of the serum

The concentration of the M component was usually determined by elution of the M com ponent separately and the value obtained was reduced by the estimated concentration of the normal serum proteins with the same rate of migration When the concentration of the M component was low ($\leq$ 0 5 g/100 ml) the value was checked visually by compari on with that of other globulin fractions In 32 cases of groups I II and III M-components with gamma mobility were not eluted separately *In 25 of* these cases the component was eluted separately on a later occa ion and the re ults thus obtained *facilitated estimation* of the concentration of the M-component found on the previous occasion In the remaining 7 cases the concentration of the M-component in 4 was low and was determined by compari on with the concentrations of other protein fractions In the remaining cases the concentration was estimated at about 1 0 g/100 ml In 32 cases of myelomatosis with an M-component with gamma mobility the com ponent was not eluted separately in the e ca es a standard value of the gamma fraction of 0 5 g/100 ml was subtracted in the calculation of the concentration of the M component

Proteinuria was demonstrated with nitric acid (Heller s test—sen itivity down to about 6 mg/100 ml) In cases of proteinuria the urine was concentrated (about 1 50) in a collodium bag (Membranfiltergе ellschaft Gottingen Germany) according to Mies (1953) before electrophoresis A light-chain component was aid to be present *when a distinct globulin fraction was found in* the urine and the quotient between this fraction and albumin in the urine was clearly higher than the M component albumin quotient in the serum

The above studies as well as all the other biochemical tests were performed at the depart ment of clinical chemistry Malmo

At after-examination in the spring of 1965 determinations of the total protein and paper electrophoresis of the serum and the urine were performed at the author s laboratory at the School of Denti try Malmo where the methods described were used Quantitative analysis of control sera showed good agreement with the values obtained at the department of clinical chemistry

Fresh sera were studied for *anticomplementary activity* in connection with routinely performed cardiolipin complement fixation test at the department of clinical bacteriology

The *serum vitamin B_{12}-concentration* was determined (Euglena gracilis) by Dr A Killander Uppsala

Bone marrow puncture was performed with Waldenstrom s (1942) instrumentarium and 0 1—0 2 ml was removed A smear was prepared with the conventional slide technique In some cases *smears were prepared with marrow* particles squashed between 2 coverglasses The bone marrow smears were stained according to May—Grunwald—Giemsa at pH 7 0 Five hundred nucleated cells were counted in each of 2 smears from each puncture in groups I II and III With but few exceptions 100 plasma cells in marrow smears from each punctate were examined In the myeloma group 200 nucleated cells were counted when the number of pla ma *cells* was 20 % or more otherwise 500 cells

CHAPTER III

SUBJECTS WITH AN M-COMPONENT OF TYPE γG OR γA (GROUP I)

With the criteria of classification described above (p 14) group I comprised 108 cases Table XIII (p 110) gives data on these cases In the table a division is made between living (62 subjects) and dead (46 subjects) and the subjects are arranged firstly according to decreasing concentration of the M components and secondly according to decreasing number of plasma cells in the bone marrow

MATERIAL

In addition to what was said above (p 14) some further comments are essential

Certain differences existed between the living and the group that had died The latter had not been examined so often In 16 cases for example electrophoresis had been done on one occasion only Nine cases had not been examined with sternal puncture and in 6 skeletal x ray had not been performed In addition the follow up in the group that had died was usually short 22 *cases were observed for less than one year* In 37 cases the only or the last electrophoretic analysis had been performed within 6 months before death in six 6 months to one year in 3 more than one year to one year and 10 months before death All of the patients who had died were examined post mortem In 17 cases one part of the skeleton was examined histologically in 25 ca es 2 parts and in 4 three parts or more In most cases 2—3 specimens were taken from *each site The specimens consisted above all of* vertebral marrow and to some extent of marrow from the femur

For the years 1958 to 1964 the number of Malmo sera analysed electrophoretically could be estimated and the number of M components found each year varied between 6 and 8 per 1 000 sera analysed The number of sera analysed per year increased and considerably more than the number of beds at the hospital The range of *indications for serum electrophoresis was thus* wider from year to year It is clear from the reasons for which the electrophoresis was requested that in group I obscure elevated E S R infectious disea es and obscure fever with or without increase of E S R were the commonest indications (54 cases) In many cases electrophoresis was requested because of an abnormally high or abnormally low value found at Kunkel s zinc sulphate test A study of "plasma protein pattern in course of acute infectious disease which included Kunkel s zinc sulphate test was in progress during the years 1956—1959 (Belfrage 1963) In 21 cases tumour was diagnosed or suspected In 5 cases electro phoresis was requested because of joint symp *toms and in others because of liver cirrhosis* systemic lupus nephrosis etc In 13 cases the electrophoresis was done in association with a study of the frequency of M components (Chapter VII) control of healthy blood donors for scientific purposes and scientific family study

COMMENTS

The division of group I into living and dead was not done throughout because it was often undesirable to split the material Division was however justified from various points of view The members of the living group were more thoroughly investigated *inter alia* with repeated haematologic and serum electrophoretic analyses and their mean observation time was longer *As will later be apparent sometimes several years* elapsed after the discovery of the M component before the diagnosis of myelomatosis could be regarded as firm That skeletal x ray was not done in all cases in the group of those who had died was compensated to a certain extent by the fact that necropsy was done That some cases were not examined with bone marrow puncture at the end of the observation period was com pensated by the fact that the bone marrow was *studied histologically* At the latter examination the volume of bone marrow examined is presum ably larger than that examined in cytological smear so there should be a better chance of demonstrating myelomatosis when the changes are only patchy

In everal respects comparisons were made between group *I and the myelomato is group* It should be stressed that uch comparisons may be sometimes misleading For a case with an M component to be included in group I it had to be after examined after one year or more if not examined post mortem Many patients with myelomato is died within one year or they were for some other *reasons not after examined* (bodyweight skeletal x ray appearance of the bone marrow etc) after one year s expectant

treatment The difference between the 2 groups would probably have been greater if such an after examination had been performed

The purpose of the somewhat complicated definition of cases of myelomatosis was to obtain a group where the diagnosis of myeloma to is was firm or very probable without at the same time having to exclude too many cases or to assign them to group I It was not possible to draw a clear line of distinction between the above mentioned groups and a few cases (above all Nos 2 and 6) where the diagnosis of myelomatosis was clinically regarded as firm or very probable were assigned to group I

With the criteria used for subgroup 3 in the myelomatosis series the diagnosis was less certain than with those used for the other groups Osteolytic lesions can be caused by neoplastic diseases other than myelomatosis (p 41) and bone marrow plasmocytosis has been found in cases of other malignant tumours (p 36) With the exception of cases 82m and 86m (66 and 22 % plasma cells) all the Malmo cases belonging to subgroup 3 had been followed up for at least one year without the appearance of signs of any coexisting malignant neoplasm

The frequency of M components detected per 1000 sera examined was largely unchanged during the period of the investigation The electrophoretic pattern was thus judged in a uniform way during the period in question

OBSERVATION TIME

Group I —Observation time is to be understood here as the interval between the discovery of the M component and the last after examination (including bone marrow puncture, skeletal x ray and serum electrophoresis) or the first electrophoretic examination that no longer showed an M component, or death The observation time was on the average 3 years (arithmetic mean) The living had been observed on the average for 3 years and 10 months and for those who had died the corresponding time was one year and 11 months Since the after examination in the spring of 1965 one year has elapsed without any of the subjects having been admitted to Malmo general hospital for myelomatosis

Myelomatosis group —The interval between the detection of the M component or the diagnosis and death or the end of this investigation (June 30 1965) was on the average 1 year and 8 months and ranged from 4 days to 8 years and 3 months (excluding 12 patients who had evidently died from some diseases other than myelomatosis, *e g* myocardial infarction, cerebral haemorrhage bleeding peptic ulcer)

The observation time and the significance of the duration of this time in the diagnosis of myelomatosis is discussed further below (p 42 46)

AGE AND SEX

RESULTS

The mean age in group I at the time of discovery of the M component was 67 years for both men and women At the end of the observation period the mean age was 70 years The youngest patient (case 39) was 25 years at the time of discovery of the M component and the oldest patient (case 104) was 90 years

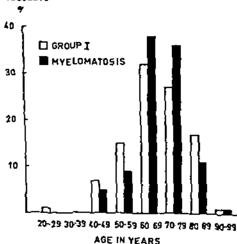


Fig 1 Age distribution at detection of M component or at diagnosis in the myelomatosis group and in group I at detection of M component

The mean age of the myelomatosis group was 69 years The age distributions

in group I and in the myelomatosis group did not differ substantially from one another (Fig 1) The cases in group I were, however, not concentrated to the same extent in the 60—79 year age class

Group I contained 52 males and 56 females The myelomatosis group included 37 males and 55 females

COMMENTS

Analysis of cases with M components but without other signs of myelomatosis and after exclusion of cases with leukaemia, lymphosarcoma or reticulum cell sarcoma (Waldenström 1952, Olhagen & Liljestrand 1955, Osserman 1958, Hammack et al 1959, Ogryzlo et al 1959, Owen et al 1959, Hurlimann & Martin 1962, Refvem & Bjornstad 1962, Stoop et al 1962, Hallen 1964, Radl & Masopust 1964, Brittinger & König 1965, Mirki & Wuhrmann 1965, Radl et al 1965) showed that the age distribution of 90 patients was largely the same as that in the material presented here Fifty eight per cent were 60—79 years and 30 % were younger (23 % in the Malmo series) The large number of aged patients agrees well with experience from a population study, which showed that the frequency of M components increases with age (Axelsson et al 1966)

Riva (1964) found that the age distribution of that part in his series which corresponded to our group I agreed with that of his total M component series In our group I (hospitalised patients and subjects found in a study of persons above 70 years of age) old persons are, however, probably over represented This assumption is supported by the following facts Judging from the results of a population study (Axelsson et al 1966) the expected number of persons with M components in the town of Malmo (230 000 inhabitants, 150,000 above 25 years of age) is about 1,200 (mean age 65 years) with the following age distribution 27 % in each of the 50—59 and 60—69 year class and 18 % in each of the 70—79 and 80—89 year class

The high mean age of cases with myelomatosis in the town of Malmö has already been discussed (Martin 1961, Waldenström 1964 c)

Analysis of some series (Adams et al 1949, Breitenbucher & Herzog 1949, Fowler & Gordon 1950, Limarzi 1951, Meacham 1953, Schwartz & Cataldo 1953, Haines & Powell 1954, Brownell 1955, Carson et al 1955, Kenny & Moloney 1956, Videbæk & Johansen 1956, Bayrd & Heck 1958, Innes & Newall 1961, Drivsholm 1964) consisting of 50 cases or more showed that of 1,583 patients, 1,020 were males In the present myelomatosis group 37 were males and 55 were females Assuming that persons from each sex are equally liable to develop myelomatosis the expected distribution for the town of Malmö would be 39 males and 53 females

Also in series of cases without myelomatosis but with M components (see above) males were more common (60 males and 30 females) This was not the case in the Malmö series (52 males, 56 females)

RED BLOOD CELL COUNT (R B C)

RESULTS

Group I —At the time when the concentration of the M component was highest subnormal R B C was common (Table XIII) Anaemia (males R B C < 4 2, females R B C < 3 8 mill) was seen in 29 % of the living and in 53 % of those who had died At that time, however, many had diseases (e g malignant neoplasms infectious diseases) which might explain the anaemia

In order to form an opinion of the R B C in largely healthy persons with M components in the serum only the living subjects were studied and the

values noted at the after examination in 1965 were u ed The values noted in 21 males and 30 females are given in

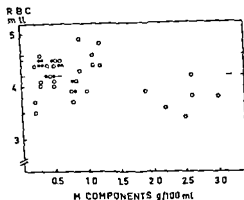


Fig 2 R B C and concentration of M-components in 51 cases in group I at last after examination (ee text) *Filled symbols males* Broken lines lower normal borders for R B C (male 4 6 female 4 2 mill)

Fig 2 (the subjects treated with melphalan were not included) Most of the males showed values slightly below what is generally accepted as the lower limit of the normal range (4 6 mill Altman 1961) while *in the females the values* were fairly evenly distributed above and below this limit (4.2 mill) Nine patients had anaemia and were therefore investigated thoroughly (Table I)

Myelomatosis group —Anaemia (males R B C < 4 2 females R B C < 3 8 mill) was noted in 76 cases (83 %) at the time of the diagno is or within one year afterwards

Of the remaining 16 (Table II) *only* 4 had M components of a concentration of more than 2 0 g/100 ml Skeletal x ray showed only a local lesion in ca es 63m 66m and 84m

Fig 3 shows the R B C and M component concentration in those cases in which paper electrophoresis had been performed In practically all cases with a component in a concentration of 3 0

Table I *Nine cases with anaemia at after examination*

Case Sex	M component (g/100 ml)	Hb (g/100 ml)	R B C (*mill*)	M C V (μ^3)	M C H C (g/100 ml)	Reticulocytes (1000)	Serum B_{12}	Serum iron (μg/100 ml)	Total iron binding capacity (μg/100 ml)	Serum bilirubin (*mg*/100 ml)	Haptoglobin (mg/100 ml)
1 F	3 2	9 8	3 0	104	32	8	normal	68	213	0 5	100
7 F	2 2	12 3	3 6	98	36	18	normal	135	330	0 3	118
23 M	1 1	12 0	3 0	108	35	22	low	50	330	0 2	200
18 M	0 8	13 3	3 9	104	34	14	normal	110	308	0 8	85
26 M	0 8	12 6	4 1	95	32	6	normal	215	323	1 1	140
31 F	0 8	11 7	3 7	98	35	10	normal	90	368	0 3	90
45 M	0 6	11 1	4 0	101	34	20	normal	200	353	0 4	165
19 F	0 2	12 0	3 7	110	33	2	normal	100	345	0 4	105
61 F	0 2	11 4	3 5	100	33	8	low	105	413	0 2	70
Normal range						10—50		55—175	260—400	0 2—1 1	30—190

Table II *Cases of myelomatosis with largely normal R B C*

Case	Sex	R B C (mill)	Bone marrow plasma cells (%)	Albumin (g/100 ml)	M component (g/100 ml)	Light[1] chain	X ray	Duration[3] (yr mth)
40m	M	4 2	5	3 0	3 1	—	+	1— 5
42m	F	3 8	11	3 7	3 0	—	—	2—10
47m†	F	4 3	28	3 4	2 7	+	+	0— 1
50m†	M	4 5	9	1 5	2 1	—		0— 1*
53m†	F	4 4		3 4	2 0	—	+	3— 4
54m†	F	4 0	23	2 7	1 9	—	+	2— 3*
55m†	F	4 3	11	4 4	1 7	—	—	5— 0
58m†	F	4 2	12	4 3	1 1	—	—	6— 3*
63m†	M	4 6	4	3 0	0 7	—	—	0— 3*
64m†	F	4 9		4 2	0 7	—		6—10
65m	F	4 3	3	2 9	0 6	—	+	3— 7
66m	M	4 7	2	5 1	0 6	—	+	1— 9
71m†	M	5 2	7	4 8	0 3	—	—	1— 8
72m†	M	4 7	3	4 6	0 3	—	—	5— 2
83m†	M	4 3	14	4 2	—		+	0— 1
84m†	F	4 0	2	3 1	—	—	+	0— 3*

[1] Light chain component in urine
[2] Osteolytic lesions
[3] Interval between diagnosis or detection of M component and death or end of this investigation
Asterisk = death from other disease

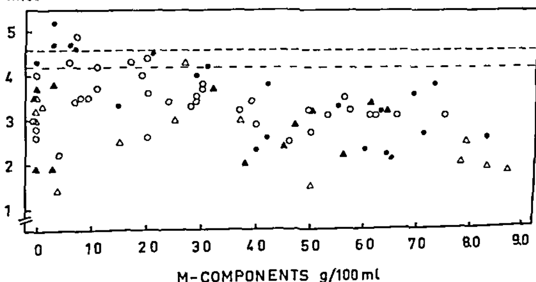


Fig 3 R B C and concentration of M components in the myelomatosis group Filled symbol males Triangles light chain component Broken lines lower normal borders for R B C (male 4 6 female 4 2 mill)

g/100 ml or more there was anaemia In 13 patients of the 24 with serum components of lower concentration and no light chain component there was no anaemia

COMMENTS

The difficulty to evaluate the finding of a low R B C in cases in group I is apparent from the fact that anaemia was seen not only in subjects with high but also in some with a low concentration of M component and by the fact that one subject (No 35) had anaemia already before an M component was demonstrable and another (No 19) a constantly low R B C though the M component seemed to be disappearing In 2 cases of the 9 specially investigated ones the anaemia appeared to be due to vitamin B_{12} deficiency Otherwise the variables studied did not reveal any unequivocal aetiological factors

In myelomatosis anaemia is common (Fowler & Gordon 1950 Schwartz & Cataldo 1953 Bayrd & Heck 1958 Mallarme et al 1959 Drivsholm 1964) In the Malmo series 83 % had anaemia Some of the other 16 were characterised by a low M component concentration only slight bone marrow plasmocytosis and protracted course and thus provided examples that the line of distinction between myelomatosis and group I can be diffuse for some time

BONE MARROW

RESULTS

Quantitative findings in group I —In 96 cases one to 6 bone marrow punctures had been performed

	Number of punctures						
	0	1	2	3	4	5	6
Living	3	4	12	31	8	3	1
Dead	9	23	9	4	0	0	1
Total	12	27	21	35	8	3	2

Sternal puncture was performed in all the cases and in 49 also puncture of the spinous process of a lumbar vertebra at the after examination in 1965 In the 3 living subjects in whom bone marrow puncture had not been performed the M component disappeared

Bone marrow smears were prepared from marrow particles ad modum Fadem & Berlin (1951) in association with 91 punctures (including 15 in subjects belonging to the γM group) About one fourth of the smears prepared in this way were judged as being richer in cell or more homogenous than conventional smears The difference was however of no diagnostic significance

Puncture of the spinous process of a lumbar vertebra was done in 56 cases (including 7 with γM component) In 16 cases (group I) the aspirate from the spinous process contained more plasma cells than the aspirate from the sternum The differences were however, slight and in no instance did the spinal marrow differ from the sternal marrow in any respect of diagnostic importance

In order to estimate the amount of blood in the preparations the neutrophilic granulocytes were counted Only cell in which one or more nuclear parts appeared to be entirely isolated or to be connected with other parts only by a fine thread were accepted as granulocytes When only the preparations from the puncture yielding the highest number of plasma cells were accepted the mean number of granulocytes was 10 0 % and the standard deviation 6 2 % The error of the method counting 1 000 cells was 1 0% Among the living where the results of the marrow puncture were usually technically more successful the mean number was 8 2% and the standard deviation 4 0 % Sixteen per cent of the total and 3 % of the latter group had more than 16 % granulocytes which was taken as an approximate limit values above which suggested a substantial admixture of peripheral blood

If bone marrow containing 10 % plasma cells and 8 % neutrophilic granulocytes is mixed with blood containing 50 % neutrophilic granulocytes *mixtures that contain e g 17 23, 29 and 34 % granulocytes* will contain 8 6 5 respectively 4 % plasma cells If the marrow contains 20 % plasma cells this number will be reduced to 16, 13 10 respectively 8 % on dilution which gives the same relative number of granulocytes as above Assuming that the blood contains 70 % *granulocytes the corresponding dilutions will reduce a plasma cell count of 10 % to 9 8 7 and* 6 % respectively and a plasma cell count of 20 % to 17 15 13 and 12 % respectively

The number of lymphocytes was on the average 15 7%, the standard deviation 7 9% and the error of the method counting 1,000 cells was 1 1 % Ten per cent of the entire material and 3 % of the living had 24 % lymphocytes or more (mean + S D)

On the occasion when the largest number of plasma cells was noted, the mean number of such cells was 4 1 % The standard deviation was 4 2 % and the error of the method counting 1,000 cells was 0 6% and 1% in the group with a plasma cell count of 10% or more The distribution of the number of plasma cells is seen in Fig 4 Fifty-four per cent of the subjects had a plasma cell count that did not exceed 3 %, 9 % of the subjects had 10% or more and the largest number of plasma cells noted was 21 %

Of 22 cases with a plasma *cell count* of more than 5 %, No 2 (11 % plasma cells) and No 6 (16 %) probably had myelomatosis In case 3 the plasma cell count increased from 3 % to 16 % in 5 years and the concentration of the M component from 1 2—3 0 g/100 ml and there, too, myelomatosis was suspected In case 68 with a component of 1 9 g/100 ml there was a plasma cell proliferation (10%) which in view of the course was regarded as malignant M components in relatively high concentration (2 8 g/100 ml) and abundant *nucleoli* in the plasma cells (Table IV) were seen in case 4 (19 %) and in case 64

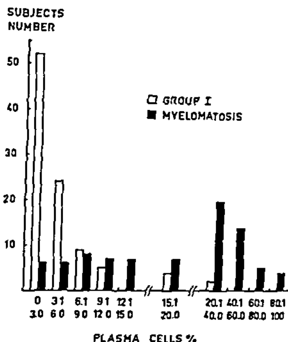


Fig 4 Highest relative number of plasma cell in bone marrow smears from cases in group I and in the myelomatosis group at diagnosis or within one year after diagnosis

(11%) A high concentration of the discrete component was noted also in cases 1 (21 %), 5 (9 %), 65 (9 %) and 67 (8 %), namely 4 1, 2 7, 2 7 and 2 0 g/100 ml respectively Marrow smears from case 1 were poor in nucleated cells Primary amyloidosis was found in cases 70 and 97 with plasma cell counts of 6 % *and 7 % respectively* Case 41 with 16 % very *atypical cells is discussed below* (p 24, 54) Case 80 with 10 % plasma cells had *polyarteritis nodosa* and case 96 with 21 % plasma cells had tuberculous sepsis and pancytopenia and an M component of *low concentration* (0 5 g/100 ml) which disappeared In the remaining 7 cases the number of cells was 6—9 % *Most of the above mentioned* cases are discussed further under the heading "Course"

The difference between punctates obtained on different occasions was more than 3 times the error of method counting 1,000 cells in 16 cases *excluding*

Table III *Cases of myelomatosis with < 10 % plasma cells in bone marrow smears*

Case	Bone marrow plasma cells (%)	R B C. (mill)	Albumin (g/100 ml)	M component (g/100 ml)	Light[1] chain	X ray[2]	Duration[3] (yr mth)
60m†	1	3 5	3 7	0 9	—	+	5—9
84m†	2	4 0	3 1	—	—	+	0—3*
66m	2	4 7	5 1	0 6	—	+	1—9
57m†	2	2 5	3 7	1 5	+	+	0—2
46m	2	3 3	3 6	2 8	—	—	4—5
72m†	3	4 7	4 6	0 3	—	—	5—2
65m	3	4 3	2 9	0 6	—	+	3—7
8m*	3	2 7	1 8	7 1			0—1
63m†	4	4 6	3 0	0 7	—	—	0—3*
56m†	4	3 3	4 6	1 5	—	+	1—0*
52m†	5	2 6	2 9	2 0	—	+	0—1*
40m	5	4 2	3 0	3 1	—	+	1—5
70m†	7	3 8	4 5	0 3	+	+	2—0
71m†	7	5 2	4 8	0 3	—	—	1—8
45m	7	3 4	4 4	2 9	—	—	8—3
79m†	8	3 2	4 1	—	+	—	0—1
44m†	8	4 0	2 6	2 9		—	0—7*
10m	8	3 1	3 8	6 6	—	+	3—1
69m	9	3 4	4 1	0 7	—	—	1—8
50m†	9	4 5	1 5	2 1	—		0—1*

[1] Light chain component in urine
[2] Osteolytic lesions
[3] Interval between diagnosis or detection of M component and death or end of this investigation
Asterisk = death from other disease

those with smears with large differences between the number of granulocyte In case 3 an increase was noted from 3 % to 16 % and the concentration of the M component increased In case 65 there was an increase from 2 % to 9 % in 7 years during which the M component increased from 1 4 to 2 5 g/100 ml the patient felt well until he finally fell ill with gastric cancer In case 80 with polyarteritis nodosa the number of cells decreased from 9 5 % to 0 5 % and the M component of 0 9 g/100 ml disappeared In the other cases the variation was insignificant or was not accompanied by any change in the course of the disease

Quantitative findings in the myelomatosis group —In this group which overlapped group I (Fig 4) 20 *cases of 84* had less than 10 % plasma cells (Table III) Local plasmocytoma of the femoral neck was thought to exist in case 65m In case 66m there were both at onset in 1963 and 1965 2 % plasma cells in the sternal marrow On the latter occasion marrow from the spinous process of a lumbar vertebra contained 12 % *plasma cells* in this case the patient had fallen ill with a picture of a local plasmocytoma of the spine As to the remaining cases an abundant admixture of blood (29—35 % granulocytes) might have been a contributory cause of the smallness of the number of plasma cells in cases 8m 46m and 63m In case 71m the course was atypical with a polyneuritis like picture and osteosclerosis In cases 45m 46m 72m 44m and 50m the M component was detected incidentally In the first 3 of these cases the course was afterwards protracted The patient in case 44m died 7 months after the discovery in hemiplegia cardiosclerosis and a metastasing prostatic cancer and No 50m died one month after the discovery

of the M-component from bleeding peptic ulcer. Case 62m appeared in the autumn of 1963 with paraplegia due to vertebral compressions, but 2 years later, after treatment with melphalan only, the patient was up and about.

Morphologic findings in group I. — In case 1 (γG) smears from punctures performed between 1961 and 1965 showed a varying number of plasma cells (21—3 %). The morphologic picture was, however, largely unchanged with some strikingly large plasma cells with loose chromatin and distinct nucleoli. A similar picture, but with still more nucleoli, was seen in case 67 (γG), see Table V.

In case 3 (γG) the plasma cells in 1959 represented 3 % of the bone marrow cells and were of normal appearance but contained nucleoli in 13 %. In 1965, when the component had increased from 1.2 to 3.0 g/100 ml, there were 16 % plasma cells including 40 % with nucleoli. In some cells both the nucleus and cytoplasm were strikingly pale. The nucleus in these cells was sometimes oval or kidney-shaped, situated centrally and had a fine chromatin and one or more small nucleoli. The cytoplasm was often scanty (Plate II: 5).

Case 4 (γG, 19 % plasma cells) showed markedly eosinophilic pellets in the vacuolised cytoplasm in about one third of the plasma cells, three fourths of which had large nucleoli (Plate I: 2, 3).

The cells in case 8 (γG, 5 % plasma cells) were strikingly small (Plate I: 8).

In case 41 (γA) with up to 16 % plasma cells, 80 % of the cells had markedly vacuolised cytoplasm with a grey structureless material in some of the vacuoles. The nuclei were dark and structureless (Plate II: 1, 2).

In case 64 (γG) with at most 11 % plasma cells, more than one fourth of the cells had nucleoli and in about half of the cells there were crystal-shaped holes in the cytoplasm (Plate I: 1).

Table IV. *Highest number of plasma cells with intranuclear inclusions, flaming or compartment formation per 100 plasma cells examined in 12 cases where such findings were made in group I (72 γG, 20 γA, 4 obscure). The figures in brackets denote: number of punctures yielding positive finding/number of punctures performed.*

Case	M component[1] g/100 ml	type	Type of plasma cell[2] a	b	c	d
7	2.5	γG			1(1/3)	
14	1.4	γG	2(1/2)		1(1/2)	
23	1.1	γG	14(2/2)			
39	0.7	γG	10(2/2)			
56	0.3	γG	6(2/4)			
58	0.3	γG	20(2/2)			
96	0.5	γG			3(1/1)	
6	2.6	γA				40(3/3)
32	0.8	γA			5(1/3)	6(1/3)
59	0.3	γA				6(2/2)
87	0.8	γA			2(1/1)	6(1/1)
93	0.6	γA		3(3/3)	1(3/3)	4(2/3)

[1] Maximal concentration, immunological type.
[2] Type a: with blue-grey, hyaline intranuclear inclusion bodies surrounded by a dark rim.
b: with other types of intranuclear inclusions.
c: with flaming.
d: with compartments.

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Table V *Plasma cell morphology in 21 cases in group I with 5 % bone marrow plasma cells or more (group A) and in 21 cases with myelomatosis but low plasma cell count (group B)*

GROUP A							GROUP B						
Case	Plasma cells (%)	Per cent of plasma cells: RE cells[1]	nucleoli[2] $\geq \frac{1}{2}$	nucleoli[2] $\geq \frac{1}{4}$	nucleoli[2] $< \frac{1}{4}$	multinuclear	Case	Plasma cells (%)	Per cent of plasma cells: RE cells[1]	nucleoli[2] $\geq \frac{1}{2}$	nucleoli[2] $> \frac{1}{4}$	nucleoli[2] $< \frac{1}{4}$	multinuclear
1	21	5		4	11	4	4m	12	2		4	8	4
96	21	6			2	0	49m	11	10		5	15	1
4	19	8	1	48	26	0	55m	11	6		5	13	0
3	16	3		1	35	0	78m	10	11		1	3	2
2	11	13		3	18	4	62m	9	9			7	4
64	11	11		3	33	3	10m	8	7		4	11	5
68	10	8	2	19	24	5	44m	8	5		12	29	2
80	10	22		3	8	4	79m	8	8		1	18	2
5	9	3			2	6	45m	7	8		7	24	1
65	9	6		1	19	8	70m	7	4		7	18	0
74	9	5		3	6	2	71m	7	2			5	5
67	8	7		33	32	2	40m	5	10		19	26	4
25	7	7			24	0	52m	5	11		5	21	1
97	7	1		1	2	1	56m	4	21	1	18	22	1
11	6	7		4	16	2	77m	3	8		3	11	1
31	6	3			16	1	65m	3	9			2	0
69	6	4		1	6	2	46m	2	10		14	30	1
76	6	3		2	8	2	57m	2	3		1	1	2
8	5	0			9	2	66m	2	9		14	5	2
16	5	3		3	3	0	84m	2	5			8	1
66	5	3		2	5	4	60m	1	2			2	3

[1] Plasmacellular reticulum cells
[2] Diameter of nucleolus/diameter of nucleus

which had 5 % plasma cells or more in the smear (group A) were compared with the 21 cases in the myelomatosis group with the smallest number of plasma cells (group B) Two cases in the last mentioned group were not included because the smallness of the number of plasma cells was judged as being due to an abundant admixture of peripheral blood Notes were made of the relative number of plasmacellular reticulum cells (Rohr 1960) of plasma cells judged visually as having nucleoli in the following size $< \frac{1}{4} \geq \frac{1}{4} \geq \frac{1}{2}$ of the diameter of nucleus and the number of multinuclear plasma cells (Table V) The number of plasmacellular reticulum cells was on the average 6 % (3 cases ≥ 10 %) in group A and 8 % (6 > 10 %) in group B The number of cases with 25 % nucleoli or more (the plasmacellular reticulum cells were nearly always nucleolated) was 10 in each group Multinuclear cells (practically all binuclear) were seen in 16 cases in group A and 18 in group B It should be stressed that group A contained some cases where the diagnosis of myelomatosis was suspected Examples are cases 2 3 and 68

In several cases it was difficult to decide from the morphology of the plasma cells whether a bone marrow smear derived from a subject with or without myelomatosis For example in case 80 with polyarteritis nodosa (Plate I 7) there were abundant *nucleolated* plasmacellular reticulum cells In cases 1 and 67 there were a number of large bare nuclei after such cells Abundant nucleoli were found in cells in cases 4 and 64 On the other hand in case 96 where *there were 21 %* plasma cells all of the plasma cells were mature The diameter of the nucleoli in cases 25, 31 and 65

of the M component from bleeding peptic ulcer. Case 62m appeared in the autumn of 1963 with paraplegia due to vertebral compressions, but 2 years later, after treatment with melphalan only, the patient was up and about.

Morphologic findings in group I. — In case 1 (γG) smears from punctures performed between 1961 and 1965 showed a varying number of plasma cells (21—3 %). The morphologic picture was, however, largely unchanged with some strikingly large plasma cells with loose chromatin and distinct nucleoli. A similar picture, but with still more nucleoli, was seen in case 67 (γG), see Table V.

In case 3 (γG) the plasma cells in 1959 represented 3 % of the bone marrow cells and were of normal appearance but contained nucleoli in 13 %. In 1965, when the component had increased from 1.2 to 3.0 g/100 ml, there were 16 % plasma cells including 40 % with nucleoli. In some cells both the nucleus and cytoplasm were strikingly pale. The nucleus in these cells was sometimes oval or kidney shaped, situated centrally and had a fine chromatin and one or more small nucleoli. The cytoplasm was often scanty (Plate II: 5).

Case 4 (γG, 19 % plasma cells) showed markedly eosinophilic pellets in the vacuolised cytoplasm in about one third of the plasma cells, three fourths of which had large nucleoli (Plate I: 2, 3).

The cells in case 8 (γG, 5 % plasma cells) were strikingly small (Plate I: 8).

In case 41 (γA) with up to 16 % plasma cells, 80 % of the cells had markedly vacuolised cytoplasm with a grey structureless material in some of the vacuoles. The nuclei were dark and structureless (Plate II: 1, 2).

In case 64 (γG) with at most 11 % plasma cells, more than one fourth of the cells had nucleoli and in about half of the cells there were crystal shaped holes in the cytoplasm (Plate I: 1).

Table IV. *Highest number of plasma cells with intranuclear inclusions, flaming or compartment formation per 100 plasma cells examined in 12 cases where such findings were made in group I (72 γG, 20 γA, 4 obscure). The figures in brackets denote number of punctures yielding positive finding/number of punctures performed.*

Case	M component[1] g/100 ml	M component[1] type	Type of plasma cell[2] a	b	c	d
7	2.5	γG			1(1/3)	
14	1.4	γG	2(1/2)		1(1/2)	
23	1.1	γG	14(2/2)			
39	0.7	γG	10(2/2)			
56	0.3	γG	6(2/4)			
58	0.3	γG	20(2/2)			
96	0.5	γG			3(1/1)	
6	2.6	γA				40(3/3)
32	0.8	γA			5(1/3)	6(1/3)
59	0.3	γA				6(2/2)
87	0.8	γA			2(1/1)	6(1/1)
93	0.6	γA		3(3/3)	1(3/3)	4(2/3)

[1] Maximal concentration, immunological type.
[2] Type a: with blue grey hyaline intranuclear inclusion bodies surrounded by a dark rim.
b: with other types of intranuclear inclusions.
c: with flaming.
d: with compartments.

Attention was focused on intranuclear inclusions, flaming or compartment formation (Paraskevas et al. 1961). The results obtained are given in Table IV. Cases with only one plasma cell with an intranuclear inclusion or 2 cells with compartment formation per 100 plasma cells examined were not included. Such findings were made on examination of 1,500 plasma cells in 15 bone marrow smears from a normal series (Gormsen 1942) courteously placed at my disposal by Prof. Gormsen.

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grouped the cells according to their degree of maturity. Characteristic of the group 'plasmocytic response' was that practically all had normal mature plasma cells. The myelomatosis group on the other hand was not so homogenous, in 17 out of 52 cases most of the cells were mature and these cases were classified as undiagnosed although many showed a clearly increased number of cells. Aherne (1958), who used a special staining technique, found a higher mean value for the size of the nucleoli in relation to the diameter of the nucleus in each of 10 consecutive cases of myeloma than in 10 cases with reactive plasmocytosis.

Quantitative findings in group I.—To assess the admixture of peripheral blood the relative number of neutrophilic granulocytes was noted. Values above 16 % were regarded as suggestive of an abundant admixture of peripheral blood but not before dilution resulting in 20—30 % granulocytes does the relative number of plasma cells decrease to such an extent as to be of practical importance. It should also be borne in mind that differential counting of bone marrow smears is a less accurate method (Segerdahl 1935 p 53) and that limits such as that given above must be regarded as approximate.

Three per cent plasma cells was said to be the upper limit of the normal range (Gormsen 1942 p 38) and about half of the cases in group I had on some occasion an increased number of such cells. Nine per cent had 10 % plasma cells or more. In 4 of these 10 cases the diagnosis of myelomatosis was however suspected. Some of the others with an increased number of plasma cells had an M component in a high concentration but the observation times were usually long and the course stationary which made the diagnosis of myelomatosis improbable. In some of the cases there were diseases such as polyarteritis nodosa, septic tuberculosis with pancytopenia, cancer and sarcoidosis which are believed to be capable of causing reactive plasmocytosis.

Quantitative findings in the myelomatosis group. – In the early stage one fourth of the cases had plasma cell counts below 10 %. In most of these cases however puncture had been performed at only one site and only once during the first year of observation. It is therefore difficult to say how often cytologically equivocal cases become clear cut within one year after discovery. The course of the concentration of the M component and the correlation between it and the number of plasma cells (p 36 and 37) suggest that only in a few cases of myelomatosis followed for a year does the number of plasma cells not exceed those generally found in group I.

Of patients with myelomatosis and a small number of plasma cells in the bone marrow most had other signs more or less strongly suggesting the diagnosis (skeletal lesions, high concentration of M component, light chain component in the urine, hypoalbuminaemia, anaemia) but in a few cases such signs were missing.

Morphological findings.—In some cases in group I the cells exhibited special peculiarities. Cells with red pellets or patches in the cytoplasm have been described previously (Streicher & Sandkuhler 1953, Heilmeyer & Begemann 1955, Axelsson et al 1966) without any relation to any particular clinical picture.

The cells of the type seen in case 41 are generally called Mott cell after the author who described them in perivascular infiltrates in the central nervous system in trypanosomiasis (1906). Stich et al (1955) distinguished between Mott cells where the cytoplasm was only vacuolised and grape cells with 'multiple globular bodies' in the cytoplasm

was much less than one fourth of that of the nucleus

In the myelomatosis group there were several cases where the appearance of the majority of the plasma cells in the smear was normal As an example mention might be made not only of cases 60m and 84m with 1 % respectively 2 % plasma cells, but also of cases 85m with 24 % and case 55m with at most 22 % of such cells (Plate I 4, II 3, 6)

COMMENTS

Differential diagnosis of plasmocytosis — *The existence of a relation between plasma cells and antibody synthesis demonstrated by Bjorneboe & Gormsen* (1941) and Fagraeus (1948) is supported by *a number of observations* (see Wuhrmann & Marki 1963, p 145), and *myeloma cell cultures have been found* to synthesise myeloma protein (Meyer 1957, 1962, Sonnet et al 1958, van Furth et al 1966)

Bone marrow plasmocytosis in myelomatosis is a common and valuable diagnostic finding (Zadek & Lichtenstein 1931, Gormsen 1942, Bayrd 1948, Mallarme & Auzepy 1962) Marrow smears from these patients without a remarkably large number of plasma cells are rare (Waldenstrom 1942, Diggs & Sturidge 1947, Adams et al 1949, Mallarme et al 1959, Innes & Newall 1961, Olmer et al 1962) One cause of a negative result is that the plasmocytosis is sometimes patchy (see Wintrobe 1961) Sometimes *positive results are obtained* when punctures are made at more than *one site (Rubinstein 1948, Ludin 1955,* Houde et al 1960, Innes & Newall 1961)

Other conditions with M *components* where plasmocytosis has been observed are macroglobulinaemia Waldenstrom (Chapter IV) and heavy chain disease (Franklin et al 1964, Osserman & Takatsuki 1964) An increased number of bone marrow plasma cells in cases corresponding to those in the present group I have been published by, among others, Osserman 1958, *Morel et al 1959,* Schobel & Wewalka 1961, Marki & Siegenthaler 1965 The *increase* was, however, usually moderate and atypia was rare

Bone marrow plasmocytosis has also been seen in conditions other than those mentioned above and has then been called "reactive" This is particularly the case in conditions with hyper gammaglobulinaemia (Bing & Plum 1937), but also in other diseases with increased activity of the immunoglobulin synthesising organ The following conditions deserve mentioning liver cirrhosis, systemic lupus, chronic and severe acute infections, rheumatoid arthritis, poly *arteritis* nodosa, serum disease and sarcoidosis (Gormsen & Heintzelmann *1941 Jarrold & Vilter 1949, Hayhoe &* Smith 1951, Klein & Block 1953, Clark & *Muirhead* 1954, Wilhelm et al 1956, Zlotnik & Karshai 1961), as well as aplastic anaemia and agranulocytosis (Clark & Muirhead 1954, Paris & Bakke 1956) and malignant tumours (Rohr 1960 p 350)

The morphology of the plasma cells is of some value in the differentiation between myelomatosis and reactive plasmocytosis In myelomatosis the cells often differ from the normal plasma cells, particularly in the following way fine sometimes structureless chromatin, which is *homogenously distributed one* or several large nucleoli, nuclei of *varying size and* shape and often abnormally large, increased nucleus/cell *relation* centrally situated nucleus, more frequently multinuclear cells, less pronounced archoplasm, and pale, structureless, peripherally unevenly outlined cytoplasm (Gormsen 1942 p 107, Bessis & Scebat 1946, Bayrd *1948,* Snapper et al 1953 p 5 Mandema 1956 p 126, Mallarme & Auzepy *1962) Fadem (1952)* examined 110 cases with reactive plasmocytosis and *52 with* myelomatosis and

grouped the cells according to their degree of maturity Characteristic of the group 'plasmocytic response' was that practically all had normal mature plasma cells The myelomatosis group on the other hand was not so homogenous, in 17 out of 52 cases most of the cells were mature and these cases were classified as undiagnosed although many showed a clearly increased number of cells Aherne (1958) who used a special staining technique found a higher mean value for the size of the nucleoli in relation to the diameter of the nucleus in each of 10 consecutive cases of myeloma than in 10 cases with reactive plasmocytosis

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He found the last mentioned cells only in cases of myelomatosis Such cells have, however, also been found in cases of polyclonal hypergammaglobulinaemia (Horster 1950, Zlotnik 1956, Lejman & Kowarz Sokalowska 1962) Electron microscopy has shown in some plasma cells a collection of amorphous material in more or less distended ergastoplasmic sacs This has been conceived as corresponding to grape or Mott cells and as Russell bodies when the changes consist of large osmophilic globules (Thiery 1958, Welsh 1962, Bessis et al 1963) In case 41, in which some of the vacuoles contained grey material, the diagnosis of myelomatosis must be regarded as unlikely

Crystal shaped deposits in the cytoplasm have been noted by Thiery (1958), who used phase contrast and electron microscopy in the examination of plasma cells from normal animals and animals that had been immunised The finding of crystals in plasma cells is thus no support for the diagnosis of myelomatosis

Paraskevas et al (1961) found plasma cells with certain properties (intranuclear inclusions flaming and compartment formation) in cases of myelomatosis with γA-components but not in cases with M components of type γG The intranuclear inclusions are mainly of 2 types, namely blue hyaline with a dark rim, and secondly inclusions with homogenous colourless material (Brittin et al 1963, Drivsholm & Clausen 1964) The blue inclusions, which Paraskevas et al (1961) did not discuss, have been observed in myelomatosis with M-components of type γA as well as γG (Brittin et al 1963, Drivsholm & Clausen 1964), in reactive plasmocytosis (Brittin et al 1963) and in cases with M components without evidence of myelomatosis (Axelsson et al 1966) In our group I blue intranuclear inclusions were found in 5 cases, all with γG components but without any other obvious features in common

Also regarding flaming cells, Drivsholm & Clausen (1964) confirmed the finding of Paraskevas et al (1961), although in a larger series single cases with γG-components sometimes showed such cells Knedel & Bödefeld (1963) found flaming plasma cells also in cases of myelomatosis *with M component of* type γG, Huhnstock et al (1964) in cases of "symptomarmen Paraproteinämien" with components of the same immunological type Brittin et al (1963) reported that they had seen such cell in a case with "reactive plasmocytosis", but they gave no case history The man (No 96) with septic tuberculosis and several flaming cells despite a γG component may be regarded as such a case In the series of Axelsson et al (1966) flaming cells were rarely seen and then only in cases with a γA-component In group I this property was not confined entirely to cases with a γA-component It should be stressed that only cells with widespread cytoplasmic eosinophilia were accepted as flaming cells Shedding cells with only patchy peripheral eosinophilia were not accepted If flaming cells are related in one way or another to the synthesis of γA globulin it would not be surprising to find such cells in normal persons even though no such cells were found *among* the 1 500 plasma cells in smears from presumably healthy persons (p 24) One should then also find fewer flaming cells than normal in patients with myelomatosis with a γG component where the concentration of γA globulin is usually low (Bachmann 1966 b), and more than expected in polyclonal hypergammaglobulinaemia ("reactive plasmocytosis")

Cells with compartment formation were abnormally common in 5 cases all with γA components, which *provides* further support for the assumption

(Paraskevas et al 1961, Drivsholm & Clausen 1964) of a relation between cells with this appearance and M components of type γA In one of the cases (No 6) it was difficult to assess the number of cells with compartment formation Transitional forms between cells with a fine network of the cyto plasm and cells with few large compart ments were seen (Plate I 5 6) and were probably an expression of a successive dilatation of ergastoplasmic sacs (Bessis et al 1963) It has long been known that thesaurocytes occur also in the absence of myelomatosis (Kabelitz 1951)

Atypical plasma cells were found in single cases in group I and not only in subjects where malignant plasma cell proliferation was strongly suspected Plasma cells of normal appearance were seen in several cases in the myelomatosis group and even in some with unequi vocal plasmocytosis One should there fore not attach too much importance to the morphology of the plasma cells in the differential diagnosis in cases with M components

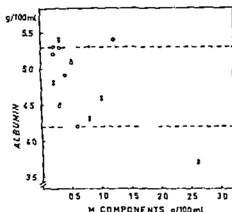


Fig 5 Concentration of albumin and M com ponents in 51 ca es in group I at last after examination (see text) Blank symbols increased concentration of alpha fraction Broken lines normal range

SERUM ALBUMIN

RESULTS

Group I —At the time of the highest recording noted for the concentration of the M component many of the patients had diseases capable of cau ing hypoalbuminaemia and hyperalpha globulinaemia (severe infections, ad vanced malignant tumours) Hypoalbu minaemia was noted at this time in 45 of the 108 patients including 28 of the 46 who had died (Table XIII) and an increased concentration of the $alpha_2$ fraction was observed in 44, including 28 who had died To assess the concentra tion of the albumin in largely healthy subjects with an M component the albumin concentrations in the living subjects noted at the after examination in 1965 were used (Fig 5 the subjects treated with melphalan were not in cluded) In 6 of the 51 subjects the concentration was below the lower limit of the normal range (4 2 g/100 ml) and then particularly among those with an M component in a relatively high con centration In 5 of 8 cases with an M component concentration above 1 5 g/100 ml the albumin concentration was decreased but only in 1 of 43 with M component concentration below this limit a difference which was statistically significant ($P < 0\ 001$)

Myelomatosis group – In 20 of 84 cases in which paper electrophoresis had been performed the albumin concentration was normal in the others the concentra tion was subnormal (Fig 6) Six of the 20 with normal values had *no serum* M component Of the remaining 14, case 66m and with some doubt also case 56m had a local plasmocytoma In cases 49m 55m 58m 64m and 72m with M components of 2 4 1 7 1 1 0 7 and 0 3 g/100 ml the course was protracted (5 to about 7 years) Of the remaining 7, 3 lived more than 1 year Three patients

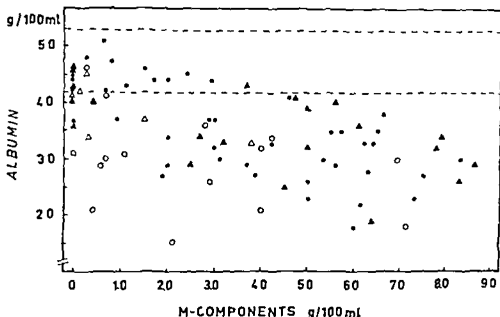


Fig 6 Concentration of albumin and M components in 84 ca es of myelomatosis Triangles light chain component Blank symbols increased concentration of the alpha fraction Broken lines normal range

(45m p 49, 51m, 61m) with M components of 2 9, 2 0 and 0 8 g/100 ml had been treated with melphalan and were living 8, 3 and 4 years after the diagnosis

In the region corresponding to an M component concentration of 3 0 g/100 ml or less, which is of interest for comparison with group I, several patients had an increased concentration of the alpha$_2$ fraction Apart from these cases and those without M component in the serum, 10 of 20 had a normal albumin concentration

COMMENTS

The ratio between the concentration of the albumin and that of the M-component has been studied by Laurell (1961) in 293 cases He found a negative but not linear correlation Accordingly most of the 51 cases studied in this respect in group I had normal albumin values Those with marked hypoalbuminaemia were only found in the group with a relatively high concentration of M component (> 1 5 g/100 ml) In sera from 12 subjects the concentration of the alpha$_2$ fraction was increased (Fig 5) The increase was, however, mostly slight and only in 4 sera was a co existing increase of the concentration of the alpha$_1$ fraction noted

In myelomatosis hypoalbuminaemia has been noted, for example, by Adams et al (1949) in 86 % Normal albumin values (> 4 2 g/100 ml) in the myelomatosis group from Malmo were seen in an early stage in 24 % Many of these cases had a concentration of the M component typical of that of group I

The possibility of deciding on the basis of increased concentration of the alpha$_2$ fraction (haptoglobin) in which cases an inflammatory reaction is responsible for the hypoalbuminaemia in the myelomatosis group is limited For in patients with a high concentration of M component hyperhaptoglobinaemia appears to be rare (Bachmann 1964) in spite of the decreased resistance to infection in myelomatosis (Zinneman & Hall 1954, Fahey et al 1963)

The inverse ratio between albumin and

M component is difficult to evaluate It appears however, that with increased globulin content an increased plasma volume may result in a low albumin concentration as well as a low R B C (Bjorneboe & Jarnum 1961)

The level of the albumin concentration seemed to be of differential diagnostic value but in the group with M components of 1 0—3 0 g/100 ml it is less reliable

GAMMA FRACTION

RESULTS

Group I —The concentrations of this fraction (paper electrophoretic gamma globulin fraction excluding any M components of gamma mobility normal range 0 8—1 3 g/100 ml) at the time when the concentration of the M component was found to be highest are given in Fig 7 In 50 cases (46 %) the concentration was below the lower limit of the normal range Low values were noted in cases with M components of both high and low concentration The number of subjects with a gamma fraction of less than 0 8 g/100 ml was, however higher ($P < 0\ 001$) among those with an M component concentration above 1 5 g/100 ml (16 of 19) than among those with a component of lower concentration (35 of 89)

In none of the 9 with increased concentration of the gamma fraction did the M component concentration exceed 1 0 g/100 ml and in 5 of them the component disappeared (Chapter VI)

In most cases the concentration of the gamma fraction was fairly constant If those cases be ignored in which the M component disappeared the difference between the highest and the lowest values recorded was not larger than 0 3 in 60 of 81 cases and 0 4 g/100 ml in 12 The largest difference (+ 1 0 g/100 ml) was found in patient 67 (Fig 16 p 41), who had *inter alia* kerato conjunctivitis sicca and rheumatoid factors in the serum and in whom necropsy showed liver cirrhosis In the other cases the variations occurred without any clear tendency or any change of interest in the rest of the picture

Myelomatosis group —Twenty five patients had either no serum M component or components differing in mobility from that of the gamma fraction The concentration of this fraction in these cases was on the average 0 5 g/100 ml and only in one case was it normal (0 9 g/100 ml) and in one lightly increased (1 4 g/100 ml 84m p 36) In 30 cases where the M component was eluted separately and the concentration of the gamma fraction

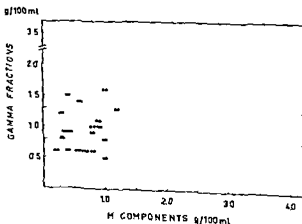


Fig 7 Concentration of gamma fraction at highest concentration of the M-components in group I Broken lines normal range

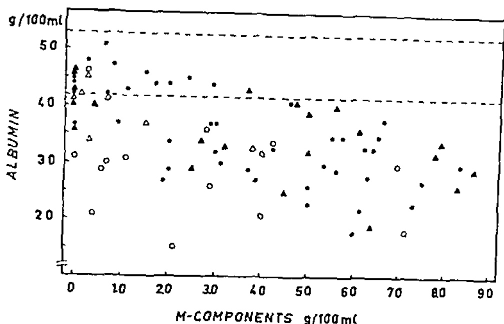


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The inverse ratio between albumin and

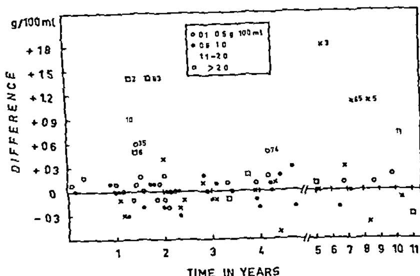


Fig 9 Differences in concentration of M-components between first and last examination in 80 cases in group I Symbols denote level at first examination Case number given in cases with large positive difference

Of those subjects with an M component concentration above 2.0 g/100 ml, Nos 2 and 6 were clinically interpreted as suffering from myelomatosis This diagnosis was suspected in case 3 at the end of the investigation period and in case 1 especially initially when the M component concentration rapidly increased to 4.1 g/100 ml

The difference between the first and the last values noted for the concentration of the M components was taken as a manifestation of the development in 80 cases (in the remaining 28 cases electrophoresis was performed only once or the M component had disappeared or shown a clear tendency to disappear chapter VI) Variations of more than ± 0.3 g/100 ml were unusual (Fig 9) An increase of 0.5 g/100 ml or more was seen in 10 cases all of which were discussed in association with the course

Only in 5 cases had electrophoresis been done before the discovery of the M component The component disappeared in cases 45a and 55 (Chapter VI) Case 35 is accounted for on page 100 In cases 51 and 107 it was difficult to decide whether an M component was present on one or more of the strips from examinations before that at which the component had been found

Fig 10 shows the relation between the number of plasma cells and the concentration of the M component in 89 cases (preparations with obviously low number of bone marrow cells were not included) The correlation coefficient (0.67) was statistically significant (P < 0.001)

The ESR (Westergren) is given in Fig 11 The figure is based on 66 cases where electrophoresis had *on some* occasion shown a normal concentration of albumin and of the alpha fraction If more than one such observation had been *made* the ESR noted at the time when the concentration of the M component was highest was used There was some relation between the ESR and the concentration of the M component The correlation coeffici-

could be estimated, it was found to be on the average 0.5 g/100 ml, and the highest value noted was 0.7 g/100 ml.

COMMENTS

The method for determining the concentration of the gamma fraction by paper electrophoresis in subjects with an M component of gamma mobility is not quite reliable (Laurell 1961). The method is, however, most reliable in subjects with M components in low concentration, i.e. corresponding to those in group I. Such a band is usually narrow and distinct, and if the concentration does not exceed about 0.5 g/100 ml it is possible to compare it visually with other globulin fractions.

In half of the cases in group I the gamma fraction was of subnormal concentration and a number crowded around the level typical of the myelomatosis group (0.5 g/100 ml). Even if low values were most common in cases with a relatively high concentration of M component ($>$1.5 g/100 ml), a low gamma fraction concentration was noted also among several cases with components of low concentration. Thus a decreased concentration of the gamma-fraction in a subject with an M component need not mean the existence of myelomatosis, a disease in which a low gamma fraction concentration is common (see Eastham & Yeoman 1960). Increased values were found in a few cases, especially cases in which the presence of the M component was only temporary and in which the disorder of the plasma cell activity thus did not appear to be so profound, an assumption supported by the fact that the concentration of the M-components did not exceed 1.0 g/100 ml.

M COMPONENT

RESULTS

Group I.—Concentrations above 1.0 g/100 ml were recorded in 32 % of the cases and values above 2.0 g/100 ml in 11 % (Fig. 8). The 12 subjects constituting these 11 % are discussed under the heading "Course" with the exception of

Case 7 (2.5 g/100 ml) was followed for 11 years. The M component, which remained constant, was discovered in association with investigation because of "pain in the entire body", for which no explanation could be offered. A high E.S.R. was known to have existed for a further previous 10 years (p. 42). On 4 occasions bone marrow puncture had shown a normal number of plasma cells.

Case 64, p. 38.

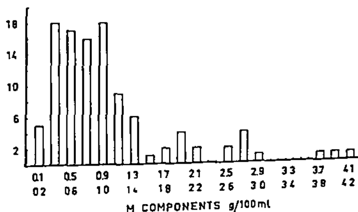


Fig. 8. Highest concentration of M components in the 108 cases in group I.

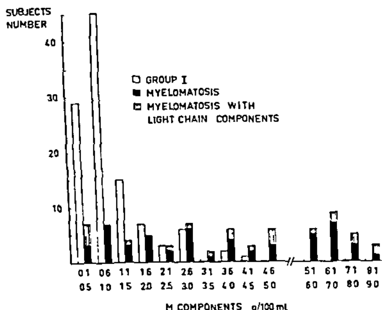


Fig 12 Highest concentration of M-components in group I and in myelomatosis group

Table VI *Cases of myelomatosis with M-components in a concentration of ≤ 2 0 g/100 ml and with no light chain component in urine*

Case	M component (g/100 ml)	R B C (mill)	Bone marrow plasma cells (%)	Albumin (g/100 ml)	X ray[1]	Duration[2] (yr mth)
51m	2 0	3 6	17	4 4	—	2— 9
52m†	2 0	2 6	5	2 9	+	0— 1*
53m†	2 0	4 4		3 4	+	3— 4
54m†	1 9	4 0	23	2 7	+	2— 3*
55m†	1 7	4 3	11	4 4	—	5— 0
56m†	1 5	3 3	4	4 6	—	1— 0
58m†	1 1	4 2	12	4 3	—	6— 3*
59m	1 1	3 7		3 1		7— 3
60m†	0 9	3 5	1	3 7	+	5— 9
61m	0 8	3 5	14	4 7	+	4— 2
62m	0 7	3 4	9	4 1	—	1— 8
63m†	0 7	4 6	4	3 0	—	0— 3
64m†	0 [illegible]	4 9		4 2		6—10
65m	0 6	4 3	3	2 9	+	3— 7
66m	0 6	4 7	2	5 1	+	1— 9
6 m†	0 4	2 2	55	2 1		0— 1
71m†	0 3	5 2	7	4 8	—	1— 8
72m†	0 3	4 7	3	4 6	—	5— 2

[1] Osteolytic lesions
[2] Interval between diagnosis or detection of M component and death or end of this investigation
Asterisk — death from other disease

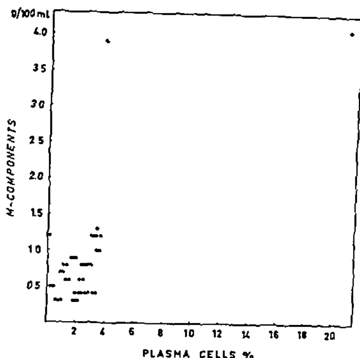


Fig 10 Correlation between relative number of plasma cells in bone marrow smear and concentration of M components $r = 0\ 67$ $P < 0\ 001$

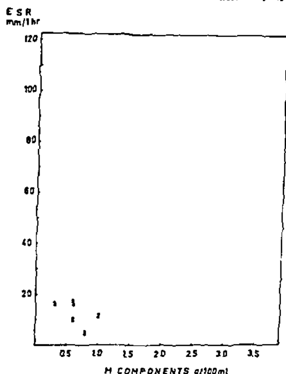


Fig 11 Correlation between concentration of M components and E S R in 66 cases in group I at the time of highest concentration of the component recorded and normal concentration of albumin and alpha$_2$ fraction $r = 0\ 37$ $P < 0\ 01$

ent was 0 38 ($P < 0\ 01$) A normal E S R (*males* < 8, *females* < 12 *mm/1 hr*) was noted in 8 cases Thirty nine per cent had an E S R not exceeding 20 mm/1 hr and several of these 26 subjects had an M component in a concentration of about 1 0 g/100 ml

Myelomatosis group —The concentration of the M component at the time of discovery or, in a few cases up to one year later are given in Fig 12, where the cases in group I are included for comparison In 73 of 84 cases examined an M component was found in the serum In 6 of 11 cases with no serum component a light chain component was found in the urine The other 5 cases are described below Several patients, including some without light chain protein in the urine had M components of relatively low concentration Thirty three (45 %) of the 73 patients had discrete components not exceeding 3 0 g/100 ml In 23 (32 %) the concentration of the component did

clinical picture was dominated by cardiac incompensation In this case serum electrophoresis was done because of an ESR of 53 mm/1 hr The concentration of the beta fractions bordered the upper normal limit which in 1954 was thought to suggest an M component Skeletal x ray showed a large osteolytic lesion in the skull which at necropsy 3 months later (the patient died from her incompensation) showed the picture of myelomatosis A bone marrow smear contained 2 % plasma cells including some in clusters and the urine contained no protein

COMMENTS

In the population study by Axelsson et al (1966) the frequency of M components increased with decreasing concentration of the components down to 0 3 g/100 ml A similar tendency appeared to exist in our group I which contained a relatively smaller number of cases with discrete components in a concentration below 0 5 g/100 ml The smallness of the number with M components in the group 0 1—0 2 g/100 ml is probably due to components of this concentration often being concealed in the normal globulin fractions

A stationary concentration of the M component was characteristic of the cases in group I The variations most frequently noted ($\pm \leq 0.3$ g/100 ml) were probably mainly a manifestation of the error of the method and were of no practical diagnostic significance If the majority of those differences shown in Fig 13 reflected a real development one would expect the differences to be greater the longer the cases had been observed No such tendency was seen

Märki & Wuhrmann (1965) studied the relation between the concentration of M component and the relative number of plasma cells in the bone marrow Their series consisted of cases of myelomatosis with M components in a concentration usually higher than those in the present group I Though the relative numbers of cells in bone marrow smears must be regarded as only approximate a statistically significant ($p < 0.001$) correlation coefficient (0 68) was found In our group I the corresponding figure was 0 67 In cases with a low M component concentration, however the calculation of a correlation coefficient between these 2 variables is less reliable because it is not possible to distinguish between the cells synthesising the M component and those synthesising the gamma fraction

The increase of the E S R depends on many factors (particularly decreased concentration of albumin and increased concentration of fibrinogen and gamma globulin) and M components increase the rate markedly (see Wuhrmann & Märki 1963 p 222) The increase of the fibrinogen concentration seen for example in infectious diseases or certain malignant tumours is often accompanied by an increase in the concentration of the haptoglobin and thereby of the $alpha_2$-fraction (Nyman 1959) Cases with hypoalbuminaemia and increased concentration of $alpha_2$ fraction were therefore not included when the increase of the E S R was studied for any relation with the concentration of the M component A positive correlation was found but in several cases with M-components also in moderate concentration (> 0.5 g/100 ml) the E S R was only slightly increased This is in accord with observations by e g Axelsson et al (1966)

The concentration of the M component in myelomatosis is not infrequently about 2 0 g/100 ml or less which appears to hold especially for type γA (Bachmann 1965) Regarding the concentrations of the M components the myelomatosis group overlapped group I mainly in the region corresponding to concentrations below 3 0 g/100 ml Special interest was focused upon cases with M components in a concentration of 2 0 g/100 ml *or less* but without a light chain component *in the urine* which finding is a strong evidence of myelomatosis (p 39) In half

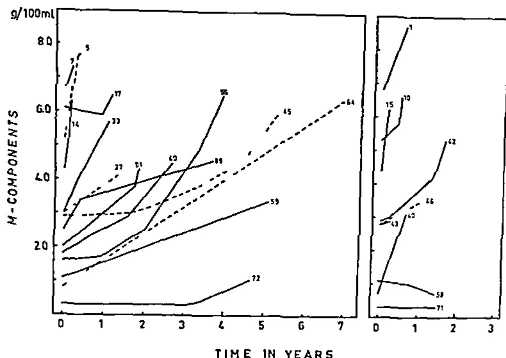


Fig 13 Course of concentration of M components in 23 cases of myelomatosis followed without cytostatic treatment Case numbers given without the letter m For clarity 2 fields and broken lines are used

not exceed 2 0 g/100 ml and in 14 (19 %) it was at most 1 0 g/100 ml

The 18 cases with discrete components not exceeding 2 0 g/100 ml and without light chain protein in the urine are given in Table VI Patients 65m and 66m had a local skeletal lesion In the latter case signs of dissemination occurred after 2 years In case 65m the M-component disappeared after treatment with roentgen radiation and melphalan and 4 years later the electrophoretic pattern was normal

In contrast to what was seen in group I, the concentration of the M-component in the myelomatosis group almost always increased with time The courses in all 23 cases where electrophoresis had been performed 2 or more times before treatment (urethan melphalan) are shown in Fig 13 In most of the cases in which determinations were made on more than 2 occasions the curve for the M component seemed to be exponential A deviating course was seen in case 71m with polyneuropathy osteosclerosis and a low constant level of the M component concentration, and in case 58m with a $_1$M component whose concentration seemed to diminish spontaneously

Only case No 56m had been examined with serum electrophoresis before the diagnosis had been established

In 5 cases without a serum M component no light chain component was demonstrated in the urine either Case 83m had proteinuria but the patient died a few days after admission to hospital and was therefore not examined further Cases 80m 81m and 82m had occasionally had slight proteinuria In all the cases Bence Jones test was performed repeatedly with negative results Only in case 81m was urine electrophoresis done It was technically unsatisfactory (1954) but nevertheless showed the occurrence of more globulin than albumin in the urine and later (after treatment) a component of 0 1 g/100 ml was shown in the serum In the above mentioned cases the concentration of the gamma fraction was low (0 3—0 4 g/100 ml) but not in case 84m (1 4 g/100 ml) in which the

clinical picture was dominated by cardiac incompensation. In this case serum electrophoresis was done because of an E S R of 53 mm/1 hr. The concentration of the beta fractions bordered the upper normal limit which in 1954 was thought to suggest an M-component. Skeletal x ray showed a large osteolytic lesion in the skull which at necropsy 3 months later (the patient died from her incompensation) showed the picture of myelomatosis. A bone marrow smear contained 2% plasma cells including some in clusters and the urine contained no protein.

COMMENTS

In the population study by Axelsson et al (1966) the frequency of M components increased with decreasing concentration of the components down to 0.3 g/100 ml. A similar tendency appeared to exist in our group I which contained a relatively smaller number of cases with discrete components in a concentration below 0.5 g/100 ml. The smallness of the number with M components in the group 0.1–0.2 g/100 ml is probably due to components of this concentration often being concealed in the normal globulin fractions.

A stationary concentration of the M component was characteristic of the cases in group I. The variations most frequently noted (≤ 0.3 g/100 ml) were probably mainly a manifestation of the error of the method and were of no practical diagnostic significance. If the majority of those differences shown in Fig 13 reflected a real development one would expect the differences to be greater the longer the cases had been observed. No such tendency was seen.

Märki & Wuhrmann (1965) studied the relation between the concentration of M component and the relative number of plasma cells in the bone marrow. Their series consisted of cases of myelomatosis with M components in a concentration usually higher than those *in the present* group I. Though the *relative numbers* of cells in bone marrow smears must be regarded as only approximate a statistically significant ($p < 0.001$) correlation coefficient (0.68) was found. In our group I the corresponding figure was 0.67. In cases with a low M component concentration however the calculation of a correlation coefficient between these 2 variables is less reliable because it is not possible to distinguish between the cells synthesising the M component and those synthesising the gamma fraction.

The increase of the E S R depends on many factors (particularly decreased concentration of albumin and increased concentration of fibrinogen and gamma globulin), and M components increase the rate markedly (see Wuhrmann & Märki 1963 p 222). The increase of the fibrinogen concentration seen for example in infectious diseases or certain malignant tumours is often accompanied by an increase in the concentration of the haptoglobin and thereby of the alpha fraction (Nyman 1959). Cases with hypoalbuminaemia and increased concentration of $alpha_2$ fraction were therefore not included when the increase of the E S R was studied for any relation with the concentration of the M component. A positive correlation was found but in several cases with M components also in moderate concentration (> 0.5 g/100 ml) the E S R was only slightly increased. This is in accord with observations by *e g* Axelsson et al (1966).

The concentration of the M component in myelomatosis is not infrequently *about 2.0 g/100 ml* or less which appears to hold especially *for type γA (Bachmann* 1965). Regarding the concentrations of the M components the myelomatosis *group overlapped* group I mainly in the *region corresponding* to concentrations below 3.0 g/100 ml. Special interest was focused upon cases with M components in a concentration of 2.0 g/100 ml or less *but without a light chain* component in the urine *which finding* is a strong evidence of myelomatosis (p 39). In half

of the cases the diagnosis was not difficult to establish (osteolytic lesions, *bone marrow plasmocytosis*) Of the other cases, some were examples of the difficulty in deciding whether an M component of moderate concentration is a sign of malignant plasma cell proliferation or not Cases with local plasmocytomas belonged of course, to the group with M components in low concentration

The relation between the size of the cell mass and its synthesising capacity has been demonstrated with mouse plasma cell neoplasms whose weight is proportional to the concentration of the M component in the serum (Nathans *et al 1958*, *Osserman et al 1964 a*) and in myelomatosis in human beings where the relative number of plasma cells in the bone marrow is correlated with the concentration of the M component (*Marki & Wuhrmann 1965*) The increasing cell mass in myelomatosis should then give rise to a gradually increasing concentration of the M component With but few exceptions *such a development was also noted in* the cases that were followed without cytostatic treatment, and in some cases with an initially slow increase the process seemed to accelerate after one or a few years

LIGHT-CHAIN COMPONENTS

RESULTS

Group 1—In 18 cases proteinuria was excluded only with the Albustix* test, or, if Heller's test was positive, urine electrophoresis was not done In 4 of these 18 cases Bence Jones test had been done with a negative result In 65 cases *Heller's* test was negative, and in 8 of these urine electrophoresis was nevertheless done but with a negative result Twenty cases had proteinuria, but no M component was demonstrable in the urine In the urine in case 19, in which Heller's test showed no protein, there were no protein fractions except a fairly broad component with the same rate of migration as that in the serum (p 40)

A light chain component was excreted in the urine *in the following 4 cases*

Case 64, in which the M component (2 6 g/100 ml) was discovered while the patient was in hospital because of thrombosis During 4 years' observation the concentration of the *component* was unchanged, no skeletal lesions were demonstrable and the bone marrow contained 11–3 % atypical plasma cells (p 24) Already at the time of the discovery there were signs of *renal injury*, which afterwards progressed The urine contained, besides a light chain component, albumin and normal globulin fractions (1–2 g/24 hr) The woman died after cholecystectomy —Necropsy increase of plasma cells in the bone marrow, pronounced sclerosis of the vessels in the kidneys, and numerous hyalinised glomeruli but nothing suggestive of myeloma nephrosis or amyloidosis

In case 68 the patient was admitted *after a short history of fatigue, vomiting* and loss of body weight, and died 2 weeks later from oliguria and uraemia The serum contained an M component (1 9 g/100 ml), the bone marrow, 10 % *plasma cells, half of which* contained nucleoli and the urine 0 03 % protein, part of which was albumin —Necropsy diffuse increase of plasma cells in the bone marrow but not sufficient to warrant a diagnosis of myelomatosis, numerous scars in the kidneys eosinophilic cylinders in the tubuli, swollen and sometimes *hyaline glomeruli, but nothing* suggesting amyloidosis

In case 74 the M component (0 5 1 0 *g/100 ml*) was *discovered while the* patient was in hospital because of *gastric ulcer* An increase of the concentration of the M component occurred at the same time as an increase of the albumin fraction *Skeletal x ray showed*

no le ions the bone marrow contained up to 9 % plasma cells and the urine a light chain component and traces of albumin and normal globulin fractions about 0 2 g/24 hr The patient died from bleeding gastric ulcer —Necropsy no increase of plasma cells no renal changes

In case 105, an M component (0 3 g/100 ml) was discovered in a frequency study The patient died soon afterwards from prostatic cancer metastasising *inter alia* to the skeleton The urine contained only a light chain component Necropsy showed no signs of myelomato sis The kidneys were not examined histologically

Myelomatosis group —In 6 cases the possibility of a light chain protein was not excluded In 10 cases positive Heller s test or the Albustix* te t (po itive or negative) had been supple mented by Bence Jones test which was negative In 49 cases there was no proteinuria or when Heller s test was positive paper electrophoresis showed no light chain component in the urine In 27 cases there was a light chain component which in 5 cases was demonstrated only by Bence Jones test These results refer above all to the time of the diagno is Only a few cases had been repeatedly controlled in this respect in the first year after establishment of the diagnosi

COMMENTS

The excretion of a light chain com ponent occurs particularly in myeloma to is and then in about half of the cases (Batts 1939 Bayrd & Heck 1947 Martin & Johnson 1957 Mallarme et al 1959 O erman & Takatsuki 1963) A po itive re ult of Bence Jones test has al o been demonstrated in urine from patients with other di eases such as lymphatic leukaemia (A kanazy 1900 Magnus Levy 1932 Rubin tein 1949 Spengler et al 1961) or malignant tu mours, particularly with skeletal meta-stases (Magnus Levy 1932, Ross et al 1937, Hughes 1954) It is true that Bence Jones test is not so very reliable (Putnam 1960) but also more exact methods have shown a light chain component in patients with skeletal metastases (Creyssel 1965) In the urine from 25 patients with a serum M component of type γG Weicker et al (1965) found a positive Bence Jones test in one ca e, a light chain protein in 6 by u e of paper electrophoresis and in 15 of 17 cases studied with immunoelectro phore is In the present material light chain components were said to be excluded if Heller s test had been negative or if paper electrophoresis of the urine had been performed

In series corresponding to group I light chain components have rarely been demonstrated in the urine (e g Märki & Wuhrmann 1963 Huhnstock et al 1964, Radl & Masopust 1964) With the methods u ed here 4 of 90 subjects were found to have a light chain component in the urine One of them like some reported by other authors had a cancer and skeletal metastases Of the other 3 No 68 presented a constellation of *clinical cytological and histological* features suggesting myelomatosis Nos 64 and 74 died from diseases which were pre umably unrelated to the serum M component They had an increased number of plasma cells in the bone marrow smears but had been followed up for 4 and 5 years without developing myelomatosis

A light chain component is a rare finding in cases of the type in group I and is accordingly of diagnostic im portance

IMMUNOLOGICAL TYPE OF M COMPONENTS

RESULTS

In 104 cases of group I the M components could be typed It was γG

in 83 cases and γA in 21 One γA-component exceeded 1 0 g/100 ml (2 6 g/100 ml in case 6 with suspected myelomatosis) In case 106 immuno-electrophoresis had not been done and in cases 19, 27 and 45 a the result of the examination was *difficult to interpret* Of great interest was case 19, where the serum and the urine contained a *heterogeneous M component in the* alpha$_2$ beta$_1$ region The serum component and the urine component reacted with anti γG globulin antiserum and with antiserum against light chain protein of type K and had a sedimentation constant of 4 0 S

Of those cases of the myelomatosis group with a serum M component and studied immunochemically the component was of type γG in 52 (75 %), of type γA in 14 (20 %), of light chain type in 2 and of type γM in one case (58m)

COMMENTS

Seligmann & Danon (1964) found that subjects with M components but without signs of myelomatosis and patients with myelomatosis did not differ in respect of the ratio between the γG and γA-components

Analysis of cases corresponding to those in group I (Creyssel et al 1959, *Hurlimann & Martin* 1962, Hallen 1964, Huhnstock et al 1964, Radl & Masopust 1964, Brittinger & Konig 1965, Marki *& Wuhrmann 1965*, Radl et al 1965) showed that 15% of 61 cases had M-components of type γA Of Osserman *& Takatsuki*s *(1963)* 68 cases corresponding to those in group I, 12 % had an M component of type γA, while the *frequency of cases with such a component* in the myelomatosis group was 29% In his large series Bachmann (1965) *found that γA components* were more common in myelomatosis (71/148) than in his groups corresponding to our group I (35/153) *Of the* serum M components found in a population study (Axelsson et al 1966) 30 % *of the 60 of* type γG or γA were of type γA The frequencies of γA-components in our *group I and in the myelomatosis group* were somewhat lower and 20 % in both, and knowledge of the immunological *type of M component* (type γG or γA) appeared to be of no diagnostic value

ANTICOMPLEMENTARY ACTIVITY (A C A)

*Serum was examined for anticomple*mentary activity in 93 subjects including all those that took part in the after *examination in 1965 Ten sera, all with* γG component, proved to have A C A

Case	Gamma fraction (g/100 ml)	M component	A C A
79	1 6	1 0	1/2560
15	0 8	1 4	>1/480
85	1 1	0 9	>1/480
5	0 6	2 7	1/480
66	0 4	2 2	1/120
10	1 2	1 9	1/60
83	1 1	0 9	1/30
31	1 0	0 8	1/15
37	0 6	0 8	1/15
39	0 6	0 7	1/10

Patients 79, 83 and 85 had liver cirrhosis Only case 79 had an increased gamma fraction concentration Only in case 5 had myelomatosis been suspected and then because of a period of increasing concentration of the M component (Fig 16, p 44)

A C A was found in sera from 13 of 86 patients in the myelomatosis group Of the 13 sera, 8 had an A C A in a titer of 1/480 or higher Twelve *had* M components of type γG one was not classified immunochemically

The findings are commented upon on page 52

SERUM CALCIUM

Hypercalcaemia was not noted in any of the cases in group I, where 91 persons were examined Apart from 4 cases

including 2 in which the M component di appeared all of the living subjects were studied at the last after examination In the myelomatosis group 70 patients were examined and 8 of them had hypercalcaemia Of these 7 had osteolytic lesions and one had advanced osteoporosis and vertebral compressions

Determination of the serum calcium is thus of no differential diagnostic value because as is apparent from the re ults set forth above, hypercalcaemia occurs mainly in advanced myelomatosis (Carson et al 1955) where the diagnosis can be based on other clinical grounds

SKELETAL X RAY

RESULTS

Group I —Roentgen examination of the skull ribs thoracic and lumbar spine pelvis and proximal femora was done in those cases seen at the after examination in 1965 with the exception of 5, where the M component had disappeared Patient 28 of the last mentioned group was the only one of the living who had never been examined Of the 46 subjects who had died 39 had been examined roentgenographically the skull, spine and pelvis in 25 two of these parts in 9 and one in 5

No o teolytic lesions were ever seen but in case 6 a cystic change was ob erved in one clavicle Puncture and exci ion of a biopsy specimen revealed no evidence of myelomato is though this diagno is was strongly suspected

Changes of the type seen *in* Paget s di ease were noted in cases 18, 102 and 107

Myelomatosis group —*In* 10 patients skeletal x ray was *not done within* the first year after the *di covery* of the M component In 56 cases x ray showed osteolytic le ions but *not in the* remaining 26 Eleven of these 26 died after so short a time that re examination could not be done one year after diagno is In the remaining 15 le ions could sometimes be demonstrated after varying periods and the observation periods were 5 years or more in 6 cases Case 71m showed patchy sclerosis

COMMENTS

Typical osteolytic lesions i e "punched out lesions' are common in myelomatosis and are thus of considerable diagnostic significance Osteolytic lesions were seen in 60—86 % of the cases in 6 series of myelomatosis representing all together 278 patients (Batts 1939, Sandkuhler 1951 Haines & Powell 1954 Kenny & Moloney 1956 Martin & Johnson 1957 Houde et al 1960) Lesions were noted at the time of the diagnosis by Andersch & Stobbe (1963) in 47 % of 58 cases and by Drivsholm (1965) in 50 % of 103 cases

In the group described here typical lesions were seen in 68 % of the cases at the time of diagnosis or within the following year Some of the remaining cases ran a benign protracted course and diagnostically important signs (*high* concentration of M *component light* chain component, grave anaemia lo s of bodyweight) were sometimes mi sing They consequently show that in a few cases of myelomato is the diagnosis cannot be established until *after* a *long* observation period

Skeletal lesions resembling tho e *in* myelomato is are al o *seen in other* conditions (Heiser & *Schwartzman* 1952 Andersch & Stobbe 1963 *Dael* 1963) These conditions are e pecially malignant *tumours* Since malignant tumours may be accompanied by bone marrow plasmocytosis (p 26) myelomatosis may be su pected in patients with a malignant tumour and o teolytic metastasis particularly if there is an M component Judging *from* the literature bone marrow plasmocytosis is fairly rare in patients with malignant tumours as are serum M components (O erman 1958) A combination *of* a tumour with osteo

lytic metastasis, bone marrow plasmocytosis and a serum M component must thus be rare. This is also apparent from the present material, in which no such case was seen in spite of the fact that serum electrophoresis is widely used at Malmö general hospital and particularly in cases treated at the department of radiotherapy.

COURSE

RESULTS

Group I. —The observation time (p. 17) often extended over several years (Table XIII and Fig. 14) and was on the average 3 years.

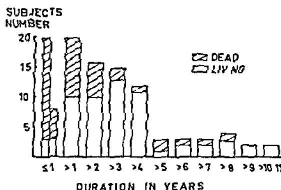


Fig. 14. Interval between detection of M component and last after-examination, first electrophoresis showing disappearance of component or death in 108 cases in group I. The class ≤ 1 year is subdivided in < ½ and > ½ year.

In 43 cases data were available on the E S R 1–22 years (mean about 8 years) before the discovery of the M-component. An M component was said to be present in the serum before it was demonstrated electrophoretically if on the first electrophoresis the component was the only possible explanation for a marked increase of the E S R (> 30 mm/1 hr) and if an obscure E S R increase of roughly the same degree was noted on several occasions before the first electrophoretic analysis. Two cases filled these criteria. In one of them (case 7) the E S R was 68 mm/1 hr in 1944, 75 in 1951, and 88, when the M-component was demonstrated in 1954. In the other (case 29) the average E S R was 37 mm/1 hr on 7 occasions between 1947 and 1950 without any tendency to increase. The E S R was on the average 43 mm/1 hr on 5 occasions after 1958, i.e. the year the M component was discovered. The E S R was determined so often because the patient was being followed up after extirpation of ovarian cancer.

The following cases call for a few comments; brief data are given in Table VII.

In some cases the diagnosis of myelomatosis was more or less strongly suspected. Cases 2 and 6 were clinically diagnosed as myelomatosis, but in accordance with the criteria used (p. 14) they were assigned to group I. In both of them the course was modified by melphalan (Fig. 15). —Myelomatosis was also strongly suspected in case 68 because of the cytologic picture of the bone marrow, the occurrence of a light chain component in the urine and the histologic findings in the kidneys, bone marrow plasmocytosis was however not so marked that the pathologist could confirm such a diagnosis. —Myelomatosis was suspected to some extent also in cases 1, 3, 67 and 74 and in one of them (case 1[1], Fig. 15) because of a primarily rapid increase in the concentration of the M component, which, however spontaneously decreased in association with improvement of the patient's general condition. —Case 3 was characterised by a steadily increasing concentration of the M component (Fig. 15) and in

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[1] The patient died in the spring of 1966 in a picture of senile cachexia. Necropsy showed a bone marrow very poor in cells with groups of plasma cells here and there which however were considered sufficient to warrant the diagnosis of myelomatosis; marked plasma cell infiltration was seen also in the lymph nodes and the spleen.

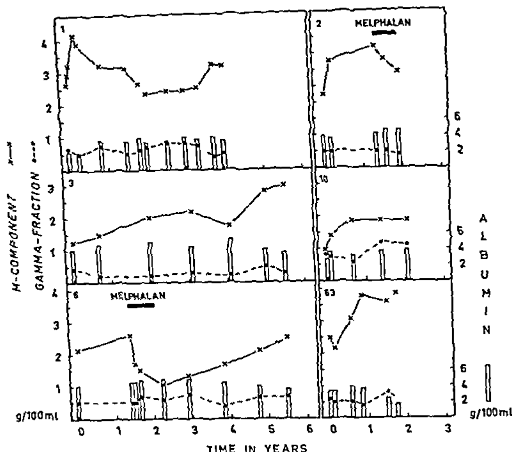


Fig 15 Course in 6 cases in group I Case number in upper left corner

creasing number of bone marrow plasma cells some of which were atypical – Case 67 was suspected because of an M component in fairly high concentration an obscure poor general condition and an increased number of atypical plasma cells in the bone marrow during the latter part of the course signs suggesting collagenosis appeared and after melphalan therapy when the concentration of the M component had somewhat decreased the gamma fraction increased markedly shortly before death (Fig 16) – In case 74 there was a light chain component in the urine and the concentration of the M *component* increased but the increase was difficult to interpret because the patient occasionally bled from a *peptic ulcer* and because the concentration of the serum albumin also increased

At check examination of cases 77 and 106 the pathologist confirmed the histological diagnosis of myelomatosis In neither case did the clinical picture lend support to this diagnosis and both had diseases (polyarteritis nodosa pancytopenia) in which bone marrow plasmocytosis can occur (p 26)

A markedly increasing concentration

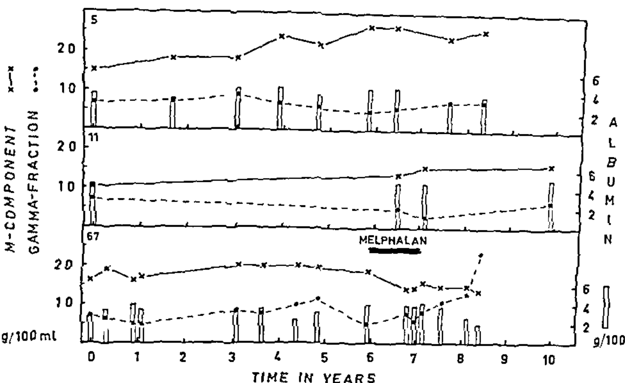


Fig 16 Course in 3 cases in group I Case number in upper left corner

of the M components was seen not only in the cases mentioned above, but also in cases 5, 10, 11, 35, 63 and 65 (Figs 15, 16 and 17) *With the exception of cases 11* and 35 the M component appeared to re*main constant after an increase of varying* duration —In case 11 the intervals between electrophoretic analyses were long, so that it could not be decided whether the course was the same in that case, too —In case 35 serum electrophoresis had been done 4 years before the first one in Fig 17 When the M component appeared, it tended to *increase rapidly*

In the subjects that had died the condition was generally dominated by symptoms of diseases in which the occurrence of an M component in the serum is difficult to interpret (p 53) A poor general condition with fatigue and loss of bodyweight was common and could usually be explained by malignant tumours, chronic infections, advanced

Table VII *Cases discussed on p 42 et seqq*

Case Sex Age	R B C (mill)	Bone marrow plasma cells (%)	M component[1] I	II (g/100 ml)	Duration (yr mth)
1F 82	3 0	21	4 1	2 6—2 5	3— 6
2F 83	3 3	11	3 7	2 3—3 7	5—11
3F 63	3 8	16	3 0	1 2—3 0	5— 0
4F 60	4 6	19	2 8	2 5—2 6	4— 8
5F 67	4 2	9	2 7	1 5—2 6	8— 4
6F 47	3 6	16	2 6	2 1—2 6	4— 9
8F 57	3 0	5	2 2	1 5—1 9	3—11
10F 73	3 8	4	1 9	1 0—1 9	1— 2
11M 48	4 6	6	1 7	1 0—1 7	9—11

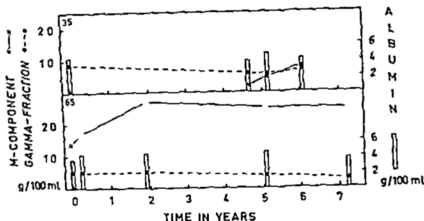


Fig 17 Course in 2 cases in group I Case number in upper left corner

arteriosclerosis amyloidosis polyarteritis nodosa or other more or less well defined diseases Besides those mentioned above subjects 66 84 87 93 and 94 are of interest because they were followed up for a relatively long time without showing signs of myelomatosis and because some of them were fairly healthy until the final stage (Table VII)

Among the 62 living subjects symptoms of various diseases were also common at the time of discovery of the M component Also at the last after examination the state of health of some

History
At discovery poor general condition rapidly increasing M-component Then protracted spontaneous improvement later deterioration
At discovery thrombosis For one year good general condition but increasing M-component and fairly many plasma cells Melphalan had good *effect* When last seen good general condition 3 % plasma cells no proteinuria
At discovery influenza Slowly increasing M-component When last seen fatigue anaemisation several atypical plasma cells
At discovery diabetic retinopathy M-component constant numerous atypical plas*ma cell* *Lost* 3 kg *in last* 3 years locomotor vision no proteinuria creatinine in serum normal
At *discovery* cholecystitis Two years later pernicious anaemia then pneumonia twice For 6 years increasing M-component then stationary When last *seen good* general condition normal plasma cell
At discovery low back pain then as later no osteolytic lesions but stationary cystic change in a clavicle Polymorphous plasma cells *Primarily increa*sing M-component development temporarily modified by melphalan When last seen good general *condition*
At discovery and later mental depression Melphalan without effect When last seen 7 years later apathy lean but M component unchanged Refused admission to hospital
At discovery bleeding *peptic ulcer* Six years previously cutaneous sarcoid nodule extirpated for a long time bronchitis x ray compatible with pulmonary sarcoidosis After rapid initial increase M-component stationary
Discovered at scientific investigation of blood donors *Slow increase* of M-component. When last seen healthy

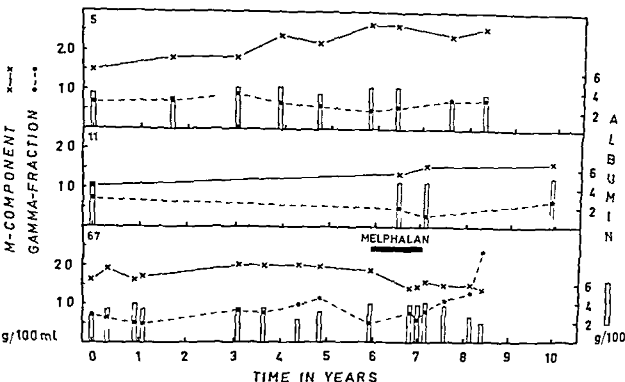


Fig 16 Course in 3 cases in group I Case number in upper left corner

of the M components was seen not only in the cases mentioned above, but also in cases 5, 10, 11, 35, 63 and 65 (Figs 15, 16 and 17) With the exception of cases 11 and 35 the M component appeared to remain constant after an increase of varying duration —In case 11 the intervals between electrophoretic analyses were long, so that it could not be decided whether the course was the same in that case, too —In case 35 serum electrophoresis had been done 4 years before the first one in Fig 17 When the *M component appeared, it tended to* increase rapidly

In the subjects that had died the condition was generally dominated by symptoms of diseases in which the occurrence of an M-component in the serum is difficult to interpret (p 53) *A poor general condition* with fatigue and loss of body weight was common and could usually be explained by malignant tumours chronic infections, advanced

Table VII *Cases discussed on p 42 et seqq*

Case Sex Age	R B C (mill)	Bone marrow plasma cells (%)	M component[1] I	M component[1] II (g/100 ml)	Duration (yr mth)
1 F 82	3 0	21	4 1	2 6—2 5	3— 6
2 F 83	3 3	11	3 7	2 3—3 7	5—11
3 F 63	3 8	16	3 0	1 2—3 0	5— 0
4 F 60	4 6	19	2 8	2 5—2 6	4— 8
5 F 67	4 2	9	2 7	1 5—2 6	8— 4
6 F 47	3 6	16	2 6	2 1—2 6	4— 9
8 F 57	3 0	5	2 2	1 5—1 9	3—11
10 F 73	3 8	4	1 9	1 0—1 9	1— 2
11 M 48	4 6	6	1 7	1 0—1 7	9— 11

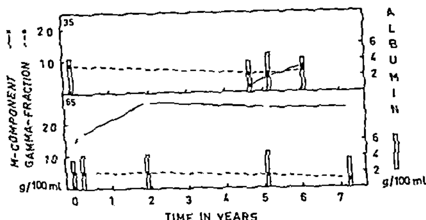


Fig. 13. Course in 2 cases in group I. Case number in upper left corner

arteriosclerosis, amyloidosis, polyarteritis nodosa or other more or less well defined diseases. Besides those mentioned above subjects 66, 84, 87, 93 and 94 are of interest because they were followed up for a relatively long time without showing signs of myelomatosis and because some of them were fairly healthy until the final stage (Table VII).

Among the 62 living subjects symptoms of various diseases were also common at the time of discovery of the M-component. Also at the last after-examination the state of health of some

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History

At discovery poor general condition, rapidly increasing M-component. Then protracted spontaneous improvement, later deterioration

At discovery thrombosis. For one year good general condition but increasing M-component and fairly many plasma cells. Melphalan had good effect. When last seen good general condition, 3 % plasma cells, no proteinuria

At discovery influenza. Slowly increasing M-component. When last seen fatigue, anaemisation, several atypical plasma cells

At discovery diabetic retinopathy. M-component constant, numerous atypical plasma cells. Lost 3 kg in last 3 years, locomotor vision, no proteinuria, creatinine in serum normal

At discovery cholecystitis. Two years later pernicious anaemia, then pneumonia twice. For 6 years increasing M-component, then stationary. When last seen good general condition, normal plasma cells

At discovery low back pain, then a later no osteolytic lesions but stationary cystic change in a clavicle. Polymorphous plasma cells. Primarily increasing M-component development temporarily modified by melphalan. When last seen good general condition

At discovery and later mental depression. Melphalan without effect. When last seen 7 years later apathy, lean but M-component unchanged. Refused admission to hospital

At discovery bleeding peptic ulcer. Six years previously cutaneous sarcoid nodule extirpated, for a long time bronchitis, x-ray compatible with pulmonary sarcoidosis. After rapid initial increase M-component stationary

Discovered at scientific investigation of blood donors. Slow increase of M-component. When last seen healthy

of the patients was not satisfactory Besides subjects 4, 8, 23, 30, 37 and 52, who were not quite healthy at last after examination and are included in Table VII, Nos 16, 22 and 44 happened to be in hospital Case 16 in which the patient was 90 years old had stumbled once or twice and was admitted on social grounds, the patient in case 22 had cystopyelitis and No 44 had acute pancreatitis Apart from those subjects all those in the living group were in a good general condition None had lost weight All were at work and nearly all of those who were pensioned off *could manage the daily activities of life*

Myelomatosis group —Of 92 patients, 76 had died Fig 18 shows the interval between the diagnosis or the finding of an *M component and death or the end* of this investigation, 12 patients who had presumably died from diseases other than myelomatosis are not included Of

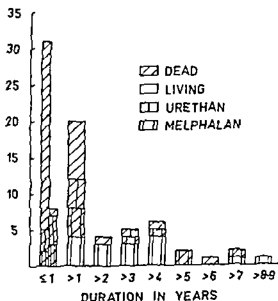


Fig 18 Interval between diagnosis or detection of M component and death or end of investigation period in 80 cases of myelomatosis The class ≤ 1 year is subdivided in ≤ ½ and > ½ year Twelve cases with principal cause of death other than myelomatosis are excluded

Table VII *Cases discussed on p 42 et seqq (cont)*

Case Sex Age	R B C (mill)	Bone marrow plasma cells (%)	M component[1] I (g/100 ml)	M component[1] II (g/100 ml)	Duration[2] (yr mth)
23 M 74	*3 0*	*4*	*1 1*	*1 0—1 1*	*3— 1*
30 F 57	4 2	2	0 9	0 8—0 8	7— 3
35 F 64	3 7	1	0 8	0 2—0 8	1— 0
37 F 68	4 4	1	0 8	0 8—0 5	2— 4
52 M 75	4 4	2	0 4	0 3—0 3	3— 0
63 F 63	2 6	4	3 9	2 5—3 9	1—11
65 M 74	4 3	9	2 7	1 4—2 5	7— 5
66 M 68	3 9	5	2 2	2 0—2 0	6— 1
67 F 63	2 9	8	2 0	1 6—1 9	8— 6
68 F 78	2 7	10	1 9		0— 1
74 M 75	4 3	9	1 0	0 5—1 0	5— 4
77 F 71	3 3	4	1 0		0 — 1
84 M 87	3 6	1	0 9	0 8—0 8	2— 7
87 M 74	4 7	3	0 8	0 4—0 6	5—10
93 M 80	3 7	3	0 6	0 6—0 6	2— 10
94 M 64	3 7	3	0 6	0 6—0 4	4—10
106 M 69	1 2	3	0 2		0— 4

[1] Highest M component concentration noted
[2] Interval between detection and last after

these 12 patients 10 died within one year Of the other 80, fifty per cent, had died within one year and one month

History

At discovery rheumatoid arthritis When last seen anaemia (pernicious) but otherwise in good condition
Discovered at routine control examination after mastectomy (cancer) Last year skeletal metastases and later pleural effusion with malignant cells cough general weakness and loss of bodyweight
At discovery pharyngitis Long history (osteomyelitis ob cure anaemia, hypothyroidism thrombophlebitis sarcoidosis (?) herpes zoster) Repeated electrophoreses without signs of M component which increased rapidly after detection
At discovery cholecystitis Since several years rheumatoid arthritis which later became more severe When last seen anaemia (Hb 11 5 g/100 ml R B C. 4 1 mill)
At discovery respiratory infection Later prostatic hyperplasia bladder catheter When last seen lost bodyweight anaemia (Hb 10 4 g/100 ml R B C. 4 4 mill) bleeding diverticulosis of colon and troubled by catheter
At discovery probably early symptoms of gastric cancer from which she died Rapid increase of M component which afterwards became stationary
At discovery cystopyelitis Afterwards in good condition First increasing M-component which afterwards became stationary Finally gastric cancer
At discovery pneumonia In good condition but cardiosclerosis On one occasion Hb 11 1 and R B C. 3 9 mill following year Hb 14 2 g/100 ml Died from ruptured aneurysm of the aorta Necropsy numerous plasma cells in lymph nodes not in skeleton
At discovery diffuse general symptoms later symptoms as in cerebral arteriosclerosis and disease of collagenous type Melphalan with slight effect on M component finally marked increase of gamma fraction Atypical plasma cells Necropsy numerous plasma cells in lymph nodes moderate number in spleen and bone marrow liver cirrhosis
Discovery after a few months fatigue and vomiting Numerous nucleolated plasma cells light-chain protein in the urine Oliguria uraemia Necropsy eosinophil cylinders in renal tubuli diffuse moderate increase of bone marrow plasma cells
At discovery as previously and later peptic ulcer often bleeding and cause of death Mostly normal plasma cells light-chain component in urine increasing serum component but also albumin Necropsy normal number of plasma cell
At discovery one month s history with vomiting urticaria fever Uraemia proteinuria no light chain Necropsy polyarteritis nodosa glomerulonephritis diffuse myelomatosis
Discovered at frequency study In good condition until death from cerebral softening Necropsy severe cardiac amyloidosis
At discovery cardiac infarction After 4 years hemiplegia but afterwards in good condition no loss of weight no anaemia Died from cerebral haemorrhage Necropsy numerous plasma cells in spleen and lymph nodes not in skeleton cardiac amyloidosis
At discovery cardiosclerosis and anaemia (not investigated further) which remained unchanged Senility but otherwise good general condition. Necropsy advanced arteriosclerosis (cause of death) local prostatic cancer
At discovery malabsorption which continued to dominate the picture (steathorrhoea hypoalbuminaemia poor B_{12} absorption despite intrinsic factor) Renal insufficiency Necropsy occlusion of iliac artery (cause of death) chronic pyelonephritis inflammation of intestinal mucosa with plasma cell infiltration low intestinal villi
At discovery pancytopenia (progressing since 15 years) psoriasis with arthropathy (since 30 and 20 years respectively) cardiosclerosis ulcerative colitis Normal plasma cell. no proteinuria Necropsy myelomatosis

(I) and concentration at first and last electrophoresis (II) examination or death.

One fifth of the patients were followed up for more than 3 years which was the average observation time in group I

Ten per cent survived more than 5 years All of the survivors had been treated with melphalan

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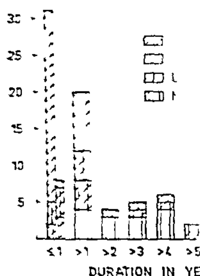


Fig. 18. Interval between diagn
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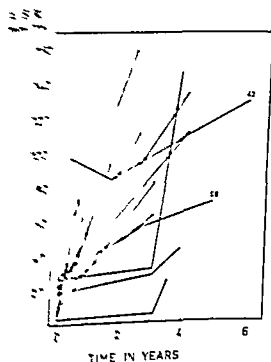
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[illegible] course of E S R before diagnosis in 14 [illegible] myelomatosis, see text Case numbers given without the letter m

These cases were selected in which data were available on the E S R up to 4 years before the electrophoretic analysis at which the M component was discovered and then showed a normal concentration of the alpha$_2$ fraction When still earlier E S R values were available they were included if they contributed to illustrate the course and the interval between such early recordings and the following recording was not more than 4 years In most cases there was a marked increase of the E S R and in many from a nearly normal level (Fig 10) Exceptions were cases 42m and 38m which were atypical particularly because of their duration

In 63 of 73 cases where an M component had been demonstrated electrophoretically had been [illegible] because of clinical picture [illegible] pneumonia which [illegible] myelomatosis [illegible] was In [illegible] E S R or d[illegible]

abnormal result of Kunkel's zinc sulphate test noted when the patients were in hospital for conditions such as cardiosclerosis, cerebral vascular lesions, peptic ulcer, melancholia

The interval between the onset of symptoms and diagnosis was one month or less in 19 cases and one year or less in 74 Of the remaining cases, No 40m had had hip pain for 2 years when skeletal x ray showed lesions of the spine, pelvis and skull and electrophoresis revealed an M component, the course was afterwards fairly rapid —Patient 18m was admitted on 2 occasions because of respiratory tract infections, no attempt was made to ascertain the cause of anaemia (R B C 3 6 mill) or the increased E S R (lowest record 46 mm/1 hr) and the diagnosis of myelomatosis was made about 2 years later —The patient in case 70m was admitted 2½ years before the diagnosis and then spent half a year in hospital because of back pain which was ascribed to an obscure compression of a vertebra, he was then in a good general condition and returned to work for 2 years before generalised pain supervened and the diagnosis was established —The patient in case 78m had for 4 years had a carpal tunnel syndrome due to amyloidosis —Patient 38m had noticed a tumour in the sternum 6 years before the diagnosis was made —In the remaining 13 cases the M component was discovered incidentally in association with care for e g cardiosclerosis, cerebral vascular lesion, peptic ulcer abdominal pain

The symptoms at onset included skeletal pain in 46 patients Of those in whom skeletal pain was not an initial symptom 3 had mucosal bleeding from the mouth 4 pneumonia 7 complained of severe fatigue 7 sought advice for anorexia and loss of bodyweight and 9 for both fatigue and loss of weight Of 71 cases in which the bodyweight had been measured before the diagnosis or

during the first year of observation loss of weight was noted in 56 (78 %) In 46 it could be estimated and was about half a kilo or more per month in 35 of them Of the 15 patients who had not lost bodyweight 2 had local plasmocytoma 3 were alive (3—8 years after diagnosis) and of the remaining 10, six survived for 2—6 years

Some cases in the myelomatosis group are remarkable and the first 3 are examples of a benign course of local plasmocytoma It was however only the last of these cases which was classified without hesitation as plasmocytoma The patient in case 38m was not examined when she first noticed a tumour and in case 66m signs of dissemination appeared about one year after diagnosis

Case 38m Female born in 1887 For 6 years she had had a nodule of varying size in the sternum when in 1954 she sought advice because of local pain Examination revealed R B C 3 2 mill 13 % plasma cells in the bone marrow a serum M-component (3 7 g/100 ml) and osteolytic lesions in the skull The histological diagnosis of the nodule in the sternum was myelomatosis She deteriorated and died 2 years later

Case 66m Male born in 1917 He fell ill in September 1962 with back pain and paraplegia R B C 4 9 mill 2 % plasma cells in the sternal marrow serum M component 0 2 g/100 ml no proteinuria X ray showed an osteolytic lesion in a thoracic vertebra and histological examination of the operative specimen showed myelomatosis The patient was given radiotherapy and melphalan and recovered Two and a half year later the concentration of the M-component was 1 0 and after a further half year 1 6 g 100 ml On the latter occasion osteolytic lesions were seen in the femora and pelvis the sternal marrow contained 2% but marrow from a spinous process of a lumbar vertebra 12% plasma cells

Case 65m Female born in 1899 In 1961 she sustained a fracture of the left femoral neck after having had pain in the left hip for one year X ray suggested a spontaneous fracture The serum contained an M-component (0 6 g 100 ml) A biopsy specimen removed in association with osteosynthesis showed the histological picture of a poorly differentiated tumour probably plasmocytoma The sternal marrow contained 3 % plasma cells Radiotherapy and melphalan were given, and 2 months later no discrete component was demonstrable Nor was any such component demonstrable after 4 years when the patient was still in a good general condition

In the following cases of myelomatosis the development was slow and some of them exemplify that it is occasionally difficult to decide whether patients with a serum M component have myelomatosis

Case 42m had an E S R of more than 80 mm/1 hr on 3 occasions 4—5 years before the discovery of the M component (Fig 19) The concentration of the M component which at the time of discovery was 2 8 g/100 ml increased (Fig 13 p 36) and the R B C decreased Melphalan was started 1½ years later After a further year osteolytic lesions appeared

Case 45m Female born in 1887 (case 131 M C in Waldenström 1961 a) An M-component (2 9 g/100 ml) was discovered in 1957 during treatment for melancholia R B C 3 4 mill 7 % plasma cells in the bone marrow E S R 34 mm/1 hr During the following 2 years the concentration of the component remained unchanged as did the R B C and the patient s general condition This was also the case in 1961 except that the concentration of the M component had increased to 3 9 g/100 ml and continued to increase exponentially until melphalan was started in 1963 (Fig 13) The R B C was then 2 7 mill the bone marrow contained 11% plasma cells and skeletal x ray which had shown nothing remarkable in 1959 and osteoporosis in 1962 now revealed osteolytic lesions of the skull The patient was still alive on June 30 1965 when the present investigation was terminated but she died from myelomatosis in August 1965

In case 55m (Fig 20) there was a long history with infections arthralgia increased E S R and heredity for disseminated lupus The M component was discovered when she was admitted because of abdominal pain interpreted as due to diverticulitis of the sigmoid colon which was later resected and found to be infiltrated with plasma cells The condition was first stationary but gradually the concentration of the M component increased the patient deteriorated and died 5 years after the

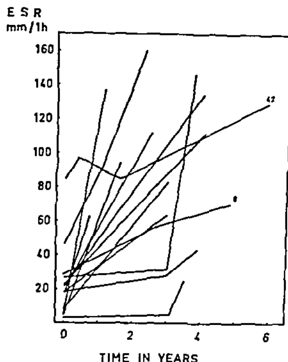


Fig 19 Course of E S R before diagnosis in 14 cases of myelomatosis see text Ca e numbers given without the letter m

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In 63 of 73 cases where an M com ponent had been demonstrated *electro-phoresis* had been performed because of clinical pictures (including *pneumonia*) which could be related to myelomatosis *In the other cases electrophoresis was* done because of a high E S R or abnormal result of Kunkel's zinc sul *phate test noted* when the patients were in hospital for conditions such as *cardiosclerosis*, cerebral vascular lesions, peptic ulcer, melancholia

The *interval* between the onset of symptoms and diagnosis was one month or less in 19 cases and one year or less in 74 Of the remaining cases, No 40m *had had hip pain for 2 years when skeletal* x ray showed lesions of the spine, pelvis and skull and electrophoresis revealed an M component, the course was after wards fairly rapid —Patient 18m was admitted on 2 occasions because of respiratory tract infections, no attempt was made to ascertain the cause of anaemia (R B C 3 6 mill) or the in creased E S R (lowest record 46 mm/1 hr) and the diagnosis of myelomatosis was made about 2 years later —The patient in case 70m was admitted 2[1] years before the diagnosis and then spent half a year in hospital because of back pain which was ascribed to an obscure compression of a vertebra he was then in a good general condition and returned to work for 2 years before generalised pain supervened and the diagnosis was established —The patient in case 78m had for 4 years had *a* carpal tunnel syndrome due to amyloidosis —Patient 38m had noticed a tumour in the sternum 6 years before the diagnosis was made —In the remaining 13 cases the M component was *discovered inci* dentally in association with care for *e g* *cardiosclerosis, cerebral vascular lesion,* peptic ulcer, abdominal pain

The symptoms *at onset included* skeletal pain in 46 patients Of tho e in whom *skeletal pain was not an initial* symptom 3 had mucosal bleeding from *the mouth 4 pneumonia* 7 complained of severe fatigue 7 sought advice for *anorexia and* loss of bodyweight and 9 for both fatigue and loss of weight Of 71 cases in which the bodyweight had been measured before the diagno is or

or proteinuria and at most 7 % mainly normal plasma cells in the bone marrow

In the following 2 cases the clinical diagnosis of myelomatosis could not be confirmed at necropsy In case 56m a local change had probably responded well to treatment at least from a histological point of view In case 78m in which the patient died from pericollagenous amyloidosis x rays had revealed osteolytic lesions of the skull and pronounced autolysis made it difficult to evaluate the histological picture of the bone marrow

COMMENTS

As is apparent from chapter I several authors have had the opportunity of following cases like those in our group I for several years and found the condition to remain unchanged and not to develop into myelomatosis Seventeen of the cases described here were followed for more than 5 years In 2 cases the observation time (time of increased E S R included) was 18 and 21 years

In some cases a diagnosis of myeloma tosis was more or less suspected Two of them were according to the criteria used, assigned to group I Often the increase of the concentration of an M component directed the examiner s thoughts to the possibility of myelomatosis This was so initially in case 1, which also showed that a high M component concentration can decrease spontaneously The increase of the concentration in case 3 did not differ essentially from that noted in case 5 during roughly the same observation time In the latter case the concentration afterwards remained constant for 3 years and the diagnosis of myelomatosis was considered unlikely In case 3 however a change was seen in the morphology of the plasma cells which with reservation for the possibility of variation in different areas of the marrow further supports the diagnosis of myelomatosis

Of interest are those cases where the concentration of the M component seemed first to rise and then to persist at a constant level In case 63 the picture was dominated throughout by progressing symptoms of cancer, while in 3 other cases the clinical picture did not change during the increase of the concentration of the M component It seems likely that these cases were observed at the time when they were rising to the level which was afterwards constant and typical of the individual case Further control examinations of case 35 are of importance to decide whether we observed the beginning of such a development

The cases in the myelomatosis group usually showed clinical pictures and courses differing clearly from that in group I That the course was usually rapid was supported by the increment of the concentration of the M component (Fig 13), by the fact that a high E S R was noted in only a few cases during the 4 year period before the first electrophoretic analysis, and by the short duration of the symptoms before the diagnosis

In the series of Feinleib & MacMahon (1960) 50 % of the cases had symptoms no more than 5 months before the diagnosis which is largely the same as that in Drivsholm s series (1964) and in the Malmö series the corresponding time for 50 % of the cases was less than 4 months

An initial symptom of *myelomatosis is* above all pain which has been noted as an early symptom in 60—90 % (Fowler & Gordon 1950 Brownell 1955 Lebon et al 1956 Mallarme *et al* 1959 Schneider & Mazabraud 1959, Waldhack 1962) *Half of the patients reported here* had skeletal pain Loss of *weight before* or within one year after the diagnosis was noted *in* 70 % of the cases where notes *had been made of the* patients body weights In the literature the frequency *of this* symptom as an initial symptom

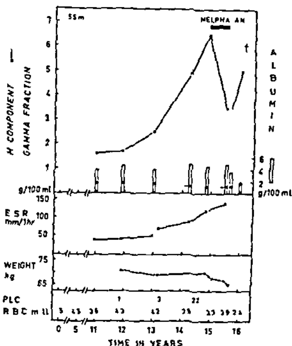


Fig 20 Course in case 55 m PLC bone marrow plasma cells

discovery of the M component —Necropsy myelomatosis, tumour forming infiltration of plasma cells in the small pelvis

Case 58m had a long history with an increased ESR and an operation because of cancer The concentration of *the M component (γM) did not increase* during an observation period of almost 2 years, after which osteolytic lesions appeared and melphalan was started She survived for a further 4 years Necropsy showed cancer metastases but not in the bone marrow, which showed the picture of myelomatosis

In cases 59m and 64m an M component was discovered in the serum 7 and 5 years, *respectively, before the diagnosis* of myelomatosis Bone marrow puncture and skeletal x ray were not done until the time of the diagnosis The concentra*tion of the M component,* which had by then increased markedly, had unfortunately not been followed in the meantime The clinical course was, however of essentially the same type as in case 72m *i e* after a long asymptomatic period rapidly progressive symptoms supervened The course was fatal in case 64m but was modified by melphalan in case 59m, in which case the patient was in a good general condition 3 years later

In case 72m (Fig 21) the M component concentration (0 3 g/100 ml) remained low for 3 years and no signs of myelom*atosis developed, apart from a slight* compression of some vertebrae Not until after almost 5 years, when back pain supervened, was the concentration of the component found to have increased and the patient died half a year later from myelomatosis

In case 71m the picture was atypical in several respects with deterioration polyneuritis like symptoms sclerotic foci in the skeleton low and constant *M component concentration, no anaemia*

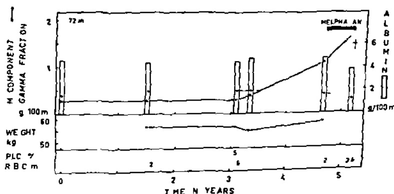


Fig 21 Course in case 72 m PLC bone marrow plasma cell

sera in group I suggests that in cases with an M component a simultaneous A C A is of no diagnostic value As in a previous investigation (Waldenström et al 1964) M components of type γG were found in all (22) sera with A C A in this extended series

There were ome ca es with a pro tracted course also in the Malmö series As in cases on record in the literature (see Innes & Newall 1961) the develop ment was similar in some ushered in by signs of local pla mocytoma In 11 of 19 other patients with a serum M com ponent followed for more than 2 years the diagnosis was however e tabli hed at the same time as the component was found

In ca e 58m the demonstration of osteolytic le ions confirmed the diag nosis The concentration of the M component at that time was still low and unchanged (about 1 0 g/100 ml) In this ca e the diagno is was question able also because the M component was of type γM which has only rarely been ob erved in myelomatosis (Adner et al 1960) —A stationary component of low concentration was also seen in ca e 71m (previou ly de cribed by Odelberg John son 1959) That patient belonged to the hitherto less well understood group with polyneuritis like symptoms and sclerotic foci in the skeleton The number of reported cases of this type (Aguayo et al 1964) is too small to know whether as in case 71m also a low concentration of the M component is characteristic

In ca es 42m 45m 46m and 55m the increase of the concentration of the M component helped to establish the diagno is and in 3 cases (42m 45m 55m) this increase seemed to accelerate Cases 59m and 64m were not followed after the demonstration of the M component but in both ca es the course was probably the same as in ca e 72m In this ca e a slight non progres ive compre ion of some vertebrae and *in some degree* a spell of herpes zoster (Davison & Balser 1937) suggested the possibility of myel omatosis a diagnosis which was not confirmed until the end of the course Thus, myelomatosis that has for years been clinically latent and protein chemically stationary can suddenly become progressive

CO EXISTING DISEASES

RESULTS

Malignant tumours before, at the time of or after, the discovery of the M component had been noted in 18 ca es in the group that had died and in 9 of the living In case 95 the tumour was a liposarcoma Patient 43 had been operated upon for melanoma 20 years before she was treated for a uterine cancer and the discovery of the M component In case 108 the patient had been operated upon for rectal cancer and breast cancer 9 years before extirpation of a melanoma, which 2 years later when the M component was discovered was metastasising Otherwise the tumours were epithelial mammary in 4 cases cutaneous in 4 including 3 with basal cell cancer pulmonary in 2 gastric in 3, prostatic in 4 including one local and *incidental* finding at necropsy ovarian in 2 uterine in one and in one the site of the tumour was the urinary bladder

In 12 cases the M component was discovered at the time the tumour began to produce symptoms Patient No 30 was operated upon in 1948 for breast cancer on one side and on the other side in 1957 when the M component was discovered Besides what was said above about cases 43 and 108 M *components* were discovered in 4 ca es 3 5 11 *and* 11 years re pectively after the *tumour* had been discovered In 8 *ca es* the tumour was discovered one year and 5 months to 7 years (*mean* 3½ years) after *di covery of the M* component In case 63 an increase was ob erved in the concentration of the M component

varies (Fowler & Gordon 1950 44%, Brownell 1955 34 % had lost more than 4 5 kg, Olmer et al 1962 about 50 %) The clinical picture is thus usually dominated early by alarming symptoms

Myelomatosis is a prognostically serious and usually rapidly progressing disease Fifty per cent survival time after the diagnosis is said to be 7—9 months (Osgood 1960, Obrecht et al 1963, Study Committee of the Midwest Cooperative Chemotherapy Group 1964) but also shorter periods (4 months) have been reported (Feinleib & MacMahon 1960) The above mentioned authors give times for 10 % survival time of about 2 to 5 years The findings in the present series agree well with these data Fifty per cent survival time was somewhat longer but would have been less if those cases were included in which the patients had died from some disease other than myelomatosis

Single cases of myelomatosis with a long duration have been described in the literature Batts (1940) observed a patient who died more than 9 years after diagnosis, Lichtenstein & Jaffe (1947), a patient who died after 9 years and one who was alive after 10 years, and Kesterson & McSwain (1952), one patient followed up for about 9 years after the diagnosis until death The patient described by Bjorn Hansen (1961) was alive 15 years after the diagnosis Some of the cases with a long course have initially presented a picture of local plasmocytoma (e g Kenny & Moloney 1957, Kaye et al 1961) The diagnoses of these cases of myelomatosis or local plasmocytoma were nearly always clear from the very beginning Kanzow et al (1964), however described a patient with myelomatosis (osteolytic lesions) who had had obscure purpura 13 years before the time when the first electrophoresis showed an M component of 2 0—3 0 g/100 ml and who was alive 13 years afterwards The development in this case was so slow that at least during the first few years after the first electrophoresis there should have been reason to doubt the diagnosis of myelomatosis In the case published by Stevens (1965) the development closely resembled that of case 72m After 6 years latency with little or no increase of the M component concentration, which from the beginning was clearly less than 1 0 g/100 ml, a change suddenly occurred with development of skeletal lesions and rapid increase of the concentration of the discrete component A similar observation has been made by Kyle & Bayrd (1966) In 1945 the patient had hyperglobulinaemia, which in 1958 (5 % bone marrow plasma cell , no osteolytic lesions) was shown to be caused by an M component (2 9 g/100 ml) In 1963 lumbar pain supervened the M component concentration was 4 6 g/100 ml, the number of plasma cells 23 %, osteolytic lesions were noted and the woman died in 1965

Of great interest are those cases in which myelomatosis developed a long time after an anticomplementary activity of the serum had suggested an M component Bloom et al (1958) reported a case with such a development after 17 years Norgaard (1964) described 5 similar cases where also electrophoresis performed at an early stage showed the occurrence of an M component Three of those with skeletal lesions must doubtless be regarded as having developed myelomatosis after 8, 13, and 17 years In one case the gamma fraction plus the M component was 1 8 g/100 ml in 1952 and was unchanged in 1956 and in 1960 it had increased to 3 7 g/100 ml That year there were 6 % and in 1962, when osteolytic lesions were first observed, 16 % plasma cells in the bone marrow

The largely equal frequency of cases with sera with A C A in group I and the myelomatosis group and the clinical picture and course in cases with such

component In cases 72, 75 and 92 necropsy showed sarcoidosis, which in cases 75 and 92 was widespread and in case 92 was also seen in the bone marrow

Skeletal changes of the type seen in *Paget's disease* were found in cases 18 102 and 107 and in all of them the alkaline phosphatase was slightly increased (10—12 U Buch & Buch) The diagnosis was verified histologically at necropsy in 2 of the cases

COMMENTS

Osserman & Takatsuki (1963 1965) and Osserman et al (1964 b) have discussed the etiology of plasma cell dyscrasia manifested by a discrete component in the serum and/or urine The high frequency of spontaneous or inducible plasma cell neoplasms in *certain inbred mouse strains* (see Osserman & Takatsuki 1965) suggested the possibility that also human myelomatosis might develop particularly in subjects with heredity for this disease Such a possibility was thought to be supported to some extent by sporadic cases of myelomatosis among relatives The possibility of hereditary factors being responsible for the development of M components also in persons without evidence of myelomatosis or macroglobulinaemia Waldenström has been suggested (Axelsson & Hällen 1965 with references to reports of relatives with one or the other of the 2 last mentioned diseases)

Osserman and his co workers thought it possible that as in Balb/c mice development of an M component required not only a hereditary but also an exogenous precipitating factor That virus could have such an effect was considered possible because virus like particles have been observed not only in the plasma cells from mouse plasmocytoma (Dalton et al 1961 Rifkind et al 1962) but also in cells from patients with myelomatosis (Sorenson 1964) That virus can produce a pronounced activity in the plasmacellular system was also considered to be supported by the polyclonal hypergammaglobulinaemia in Aleutian mink disease The protein chemical manifestation of this disease has since sometimes proved to change its character i e the hypergammaglobulinaemia assumes a monoclonal form (Porter et al 1965) The assumption that the development of M components can be induced by a variety of diseases was supported by a number of patients with chronic infections particularly of the biliary and urinary tract, and malignant tumours The authors underlined however that it was difficult to exclude the possibility of the coexistence of a disease and an M component in the serum being due solely to chance

Axelsson et al (1966) found M components in 1% of a population above 25 years and in increasing frequency with age The majority of the subjects with M component were apparently healthy or had diseases which were presumably not related to any disturbance in the immunoglobulin synthesis M components normally occurring must accordingly sometimes be found by electrophoresis of sera from diseased persons In patients with malignant tumours for example or infectious diseases or collagenoses the serum is particularly often studied electrophoretically because of a coexisting increase of the E S R This is one possible explanation for M components being often observed in these diseases Since like the M components malignant tumours are found especially in the aged the chance of discovering and M component in patients with neoplasms is surely particularly high

A perusal of a number of published series of patients with M-components irrespective of immunological type but with the exclusion of those with proved myelomatosis macroglobulinaemia Waldenström leukaemia lympho-

(Fig 15, p 43) in association with deterioration of her general condition owing to the tumour An increase was noted also in case 65 (Fig 17, p 45), but afterwards the concentration remained steady for more than 5 years, and it was not until then that the gastric cancer began to produce symptoms Otherwise in the few cases where electrophoresis had been performed more than once there occurred no variation in the concentration that could warrant any conclusions concerning any relation between the tumour and the M component In case 15, for instance, the concentration of the component had been largely constant for 4 years before a colonic tumour produced symptoms and was extirpated, half a year later the M component was unchanged

More or less chronic *inflammatory conditions were observed in some cases* In the group of living, patients 5 and 16 had had biliary symptoms for half a year *and 3 years respectively, before the* discovery of the M component In the group that had died the patient in case *64 had had biliary symptoms now and* then for 7 years before the discovery of the M-component, and in cases 78 and 79 *necropsy* showed signs of chronic cholecystitis

A history of recurrent urinary tract infections was not given by any of the living At the last after examination only case 56 (of 53 cases) showed an increased serum creatinine concentration (1 8 mg/ 100 ml) In the group that had died there were 9 cases (63, 66, 67, 70, 73, 76, 82, 94, 107) with a history of urinary tract symptoms ranging from a single attack of cystopyelitis to repeated attacks of cystitis or cystopyelitis or symptoms of prostatic hyperplasia or malignant neoplasms of the urinary tract In these cases necropsy showed signs of pyelonephrit healed or active

Single ep odes of respiratory tract infecti n had occurred in many cases (Table XIII) *Data on frequent symp* toms of this type were available in 5 cases (8, 9, 14, 32, 71) The tendency to *infection was the main clinical problem* in cases 9 and 32

Of other infectious diseases, mention *may be made of osteomyelitis in cases* 35, 59 and 99 In case 35 electrophoresis had been done on some occasions before *the appearance of the M component and* 10—20 years after the last spell of osteomyelitis —The M component in *cases 42 and 54 was discovered while the* patients were in hospital for glandular fever —The picture in case 55 suggested *encephalitis (Chapter VI) —Protracted* symptoms because of diverticulitis had been noted in case 91 —In case 96 the *patient had widespread tuberculosis*

Case 41 with serious progressive symptoms of the central nervous system *and increased total protein but no* increased number of cells in the C S F was difficult to classify diagnostically *The relation, if any, between* the occurrence of M component and the atypical cytological picture of the bone *marrow*, on one hand, and the clinical picture on the other was obscure The M component was demonstrated in an early stage of the disease and remained unchanged during progression and re gression After a long period of severe symptoms an improvement was noted when melphalan therapy was started Some regression of the E E G changes was, however noted already before the beginning of the treatment

Patients 23 and 37 had *rheumatoid arthritis* Nos 77 and 80 *polyarteritis nodosa* and Nos 67, 75, 79, 83 and 85 *liver cirrhosis*, which in Nos 79 83 and 85 produced clinical symptoms while in case 75 it was only suggested histolog ically *Sarcoidosis*, which produced clin ical symptoms, was noted in cases 10 35 and 57 *Case 35 had no* serum M component at the time of the diagnosis 5 years before the finding of the

present in some of the cases in group I. Forty per cent of the subjects above 20 years examined post mortem in Malmö (males 25 %, females 55 %) had gallstone or had been operated upon for this disease (Linell 1966). Thus the frequency of inflammation of the biliary tract in group I, which consisted mainly of hospital patients, does not appear to be remarkably high. Signs of active or healed pyelonephritis were seen in a number of cases but hardly remarkably often, since the mean age was high, which predisposes to obstructive diseases of the urinary tract (cancer of the bladder or prostate, prostatic hyperplasia).

Compared with infectious diseases and malignant tumours, the conditions classified under the name of autoimmune diseases (see Mackay & Burnet 1963) are often accompanied by more pronounced signs of an increased activity in the immunoglobulin synthesising organ in the form of bone marrow plasmocytosis (p. 26) and/or polyclonal hypergammaglobulinaemia (Waldenström 1960–61). Just as a relation is suspected between the occurrence of M components and the 2 first mentioned conditions, one might expect M components to develop also in such diseases as liver cirrhosis, systemic lupus, rheumatoid arthritis, polyarteritis nodosa. In group I, too, there were some patients with cirrhosis, rheumatoid arthritis and polyarteritis nodosa, and some with angeitis and discrete component other than the 2 in group I with polyarteritis nodosa have been observed at the department of internal medicine in Malmö (Waldenström 1966). It was, however, difficult to say anything about any causal relation between M component and the actual diseases. As to liver diseases it can be said that only one of 208 patients with cirrhosis and examined with serum electrophoresis (Hällen & Krook 1963) had an M component. This is a lower frequency than expected, especially since the majority of the patients in the cirrhosis group were over 50 years. A comparison between the cases in the population study by Axelsson et al. (1966) and the cirrhosis group is, however, somewhat misleading. The concentration of the M components in the sera from the former series was often low (about 0.5 g/100 ml or less) and such components, if any, were probably concealed by the polyclonal hypergammaglobulinaemia which was common in the cirrhosis group.

Three of 101 patients examined roentgenographically in group I showed a picture of Paget's disease of bone, as did one who was not included in group I because necropsy had not been done (case 4 B, chapter VII). The frequency was not higher than that expected from a recent review (Nagant de Deuxchaisnes & Krane 1964). Some authors, however, feel that geographical differences in frequency exist (Lackey 1960, Barry 1961) and in Sweden this disease seems to be rare (Waldenström 1966).

AMYLOIDOSIS

RESULTS

Pericollagenous amyloidosis (Missmahl 1959, Heller et al. 1964) was seen in 5 cases (70, 84, 86, 87, 97). In cases 70 and 97 it produced clinical symptoms in the form of cardiac incompensation and deterioration, respectively, the nephrotic syndrome. In these 2 cases the deposits were widespread. In the other cases they were localized mainly to the myocardium and in case 86 to the kidneys. In 37 cases routine histological examination (haematoxylin eosin) of the myocardium had revealed no evidence of amyloidosis. The type of M component was γA in case 87 and γG in the others.

COMMENTS

Osserman et al. (1964 b) reported a large series of amyloidosis and practically all of the cases had M components in the serum and/or urine. The cases

sarcoma reticulum cell sarcoma or related diseases revealed 110 case (Waldenstrom 1952, *Olhagen & Liljestrand 1955* Osserman 1958 Creyssel et al 1959 Hammack et al 1959 Ogryzlo et al 1959, Owen et al 1959 Schobel & Wewalka 1961, Hurlimann & Martin 1962 Refvem & Bjornstad 1962 Marki & Wuhrmann 1963 *and 1965 Hallen 1964 Rádl* & Masopust 1964 Brittinger & Konig 1965 Radl et al 1965) Sixteen subjects were healthy or the component was discovered in association with hospitali ation because of diseases which can hardly have anything to do with the development of M components (cerebral and cardiac va cular diseases gastritis peptic ulcer etc) Twenty one *patients had cancer Of these the site of the* tumour was the digestive tract in 5 the liver and biliary system in 2 the urogenital system in 4 the breast in one the lungs in 3 possibly 4 and the thyroid in one In the other cases the locali a tion was not given Osserman & Takatsuki (1963) and Bachmann (1966 a) have al o reported the sites of the tumours in their large series of *patients with M components The above data* allow no conclusion about the variation if any of the frequency of M components with the site of the tumour

In 8 cases there was a hi tory of repeated pulmonary infections and pulmonary em physema Diffuse hepatocellular damage but with a more or less firm diagnosis of cirrhosis was noted in 10 *cases and rheumatoid diseases* and conditions interpreted as disseminated lupus in 9 Skeletal changes of the type seen in Paget s di ease were noted in 4 cases Other diagnoses found were dermatitis pemphigus purpura asthma silicosis haemolytic anaemia (γG) etc

In group I of the Malmo series 35 % of the group that had died had malignant tumours at necropsy The figure may appear high, but the expected frequency of such tumours in a necropsy series (above 5 years of age) at the Institute of Pathology in Malmo is 42 % (Ohlsson & Norden 1965) Osserman (1958) found serum M components in 7 of 2,000 sera from patients with malignant tumours, *a frequency which does not* exceed that expected (Axelsson et al 1966) That the frequency of malignant tumours was not higher than that expected does not, of course, exclude the possibility of a connection between the M component and the tumour in some of the patients

The concentration of the M component in case 63 increased to a high level in association with progression of the *tumour As previously mentioned*, one must sometimes encounter patients cor responding to those *in group I during* the period the M component is develop ing to the level which is afterwards characteristic of the individual case On the other hand, in the above mentioned case the concentration reached was remarkably high, which supports the assumption of a relation between tumour and M component, especially since the tumour was found to be surrounded by numerous plasma cells, a finding made *in 12 cases by Osserman & Takatsuki* (1963)

Smith (1957) and Osserman (1958) believed the frequency of chronic pul monary diseases to be strikingly high among patients with M components, and Hammack et al (1959) described 3 cases with recurrent and severe infectious diseases (pneumococcal sepsis and pneu monia) A decreased resistance to in fection corresponding to that seen in myelomatosis (Zinneman & Hall 1954 Fahey et al 1963) appears to be a *possible explanation in view of the many* subjects with a low concentration of gamma fraction noted in group *I Only* in 2 cases (9 and 32) in this group did the clinical picture in some *degree* resemble that described by Hammack et al (1959) Cases 9 and 32 had not however, a lower gamma fraction con centration than many others in group I and not on the level (*≤ 0 5 g/100 ml)* usually found in cases of the "Antibody *deficiency syndrome*" (Barandun et al 1959)

On the other hand some authors feel that infectious diseases may be the cause of the *development* of an M component (Riva 1964 "Begleitparaproteinamie) Osserman et al (1964 b) and Osserman & Takatsuki *(1965) attached much* importance to the occurrence of pro tracted *infections of the* biliary and urinary tract Signs of such diseases were

present in some of the cases in group I Forty per cent of the subjects above 20 years examined post mortem in Malmö (males 25 %, females 55 %) had gall stone or had been operated upon for this disease (Linell 1966) Thus the frequency of inflammation of the biliary tract in group I which consisted mainly of hospital patients does not appear to be remarkably high Signs of active or healed pyelonephritis were seen in a number of cases but hardly remarkably often since the mean age was high which predisposes to obstructive diseases of the urinary tract (cancer of the bladder or prostate prostatic hyperplasia)

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COMMENTS

Osserman et al (1964 b) reported a large series of amyloidosis and practically all of the cases had M components *in* the serum and or urine The cases

with amyloidosis in the Malmo series have been described elsewhere (Hallen & Rudin 1966) Suffice it here to mention that also 2 cases in the group with ΓM components (XXI and XXII) had amyloidosis and that in all cases the amyloid was of peri collagenous type, *i e* of the type seen in myelomatosis and so called primary amyloidosis

CHAPTER IV

SUBJECTS WITH AN M-COMPONENT OF TYPE γM

Since Waldenström (1944 1948) described a number of cases with an abnormally high concentration of macroglobulin in the serum and several clinical features in common many similar observations have been made According to Olmer et al (1960) as many as 150–200 cases had been reported

Surveys of what has been called macroglobulinaemia Waldenström have been published with descriptions of the clinical cytological protein chemical, and histological findings (Mandema 1956 Imhof 1958 Kappeler et al 1958 Waldenström 1958 a Streiff 1959 Olmer et al 1960 Waldenström 1965) In addition the cytological picture has been described in detail by *e g* Undritz (1957) and the histological picture by Lennert (1955) Leibach (1957) Zollinger (1958) and Dutcher & Fahey (1959) Jahnke & Scholtan (1960) analysed the results obtained on ultracentrifugation

The condition is characterised by an abnormal proliferation of cells that produce macroglobulin This leads to a characteristic clinical picture The cell proliferation (mostly lymphoid sometimes more or less plasmocytic) is reflected in cell infiltration in several organs particularly the bone marrow lymph nodes spleen and liver sometimes causing enlargement of the 3 last mentioned organs In contrast to what is seen in myelomatosis skeletal lesions are rare The abnormal production of protein is reflected by an M component (γM) in the serum electrophoretic pattern sometimes also by a light chain component in the urine Mucosal bleeding eye symptom such as bleeding and venous stasis in the fundi as well as neurological symptoms such as vertigo impairment of hearing a polyneuritis like picture with pain and numbness of limbs and disorders of consciousness are probably due to the increase of the viscosity of the serum seen in some cases with a high concentration of M component (Waldenström 1944 1958 a, Fahey et al 1965) The neurological signs have also been related to cell infiltration in the C N S (Bichel et al 1950) where the cell infiltration can assume the character of a tumour (Dutcher & Fahey 1959) and where the vascular walls are sometimes swollen and P A S positive (Zollinger 1958) As in myelomatosis, the level of normal immunoglobulins is reduced and the resistance to infections is decreased (Fahey et al 1963) The serious character of the disease is also reflected in loss of bodyweight fatigue and grave anaemia

Kappeler et al (1958) *distinguished* a malignant form and a *rare benign* form A wide variety of clinical *pictures* and courses have however been observed and the borderline between malignant and benign cases *is diffuse* (Waldenström 1965, Seligmann 1966)

While numerous cases with γG- or γA component but without demonstrable signs of myelomatosis have been described *only a few* had been reported with γM components in which the diagnosis of macroglobulinaemia Waldenström had not been made or no signs of other lympho reticular neoplasms had been found (*e g* Hurlimann & Martin 1962 Rádl & Masopust 1964 Riva 1964 Seligmann et al 1965) Among 95 cases with γM components

Bachmann (1965) found 39, which could *not be assigned to the groups macroglobulinaemia Waldenström or other lympho reticular diseases, myelomatosis* or cancer (some of the cases are included *also in this chapter) Three of the 39 had* M components in a concentration of *more than 5 0 g/100* ml and *2 of* these cases were followed for more than 10 years *On the other hand,* Kanzow et al (1964) classified all their 111 cases with *γM components as macroglobulinaemia* Waldenstrom

Below an account is given of cases with γM components found in Malmo in *the course of the present investigation,* with the exception of one with myelomatosis *(Chapter III) and 8 with leukaemia,* lymphosarcoma or reticulum cell sar*coma (Chapter V)*

AGE AND SEX

In the present group of 27 cases (13 males, 14 females), including 14 in which the patients had died, the mean age was 71 years

PERIPHERAL BLOOD

RESULTS

Anaemia (male R B C < 4 2, female *R B C < 3 8 mill) was noted* in 16 cases at the time when the concentration of the M component was highest (Table VIII) The following findings were made in these patients (Figures in brackets after the case number gives the number of red blood cells in mill /mm³) Gastro intestinal bleeding was demonstrated in case XIX (4 0) with malignant cholangi*oma* and *case* XXVII (1 7) with a gastric cancer Hyperhaemolysis (*inter alia* in the form of decreased hapto globin concentration and increased carboxyhaemoglobin) was noted in case XIII (3 6) with malaria and in case II (3 5) with cold agglutinins, which were also demonstrated in case XXII (4 0) In case XXIV *(4 0)* no signs of haemorrhage, but reticulocytosis (80,000/mm³) and increased carboxyhaemoglobin were noted In cases IV *(3 8)* and V *(3 5)* there were co existing diseases which might have caused the anaemia (florid rheu matoid arthritis, chronic pyelonephritis) In 6 cases (XIV, XV, XVII, XVIII, XIX and XXV) the data available did not permit any conclusions concerning the aetiology of the anaemia In cases XVII and XIX there were, however, malignant tumours In case I *(3 6)* and case XVI (2 7) macroglobulinaemia was the only explanation available for the anaemia Case XII (p 68) had at the *first examination a Hb of 15 0 g/100 ml* and R B C 4 2 mill At after examina*tion 2 years later there was a macrocytic* anaemia (Hb 13 6, R B C 3 8, M C V *109 μ³) without signs of hyperhaemolysis* (reticulocytes 2,000/mm³, serum bili*rubin 0 3 mg/100 ml, haptoglobin 145* mg/100 ml and normal concentration of *alpha₁- and alpha₂-globulins)* and normal concentration of the serum vitamin B_{12}

Atypical cells of the same type as those in the bone marrow were found in *the blood in cases* XIV *and* XXII Case XXIV was the only one with *a thrombocyte count* below 100,000 mm³

COMMENTS

Hb ≤ 70 % was reported in 86 % of 78 cases of macroglobulinaemia Walden ström collected from the literature by Imhof (1958, p 39) He believed the anaemia to be due to causes such as inhibited erythropoiesis, bleeding and hyperhaemolysis and noted an increased fragility of the cells, which was possibly due to the abnormal *protein* Similar results have been reported by Cline et al (1963) *in erythrokinetic studies*

Several patients in the Malmö series had anaemia *In only a few, however,* could the anaemia be regarded as related to the macroglobulinaemia Two of the 4 patients with signs of hyperhaemolysis had high titre cold agglutinins These

were presumably reflected in the M components found as in the cases reported by Christenson & Dacie (1957) and Fudenberg & Kunkel (1957)

BONE MARROW

RESULTS

Bone marrow smears were available from 20 patients (Table VIII) The number of lymphocytes (i e lymphocytes and lymphocyte like cells) was 20 % or more in 9 cases and in 5 it was 30 % or more In cases II and XXII both with an increased number of mast cells and a lymphocyte count of 18% and 17% respectively, these figures were not representative because there was marked normoblastosis In none of these 11 cases was there reason to suspect an abundant admixture of peripheral blood judging from the density of nucleated cells in the smears and the number of neutrophilic granulocytes

The nuclear structure of the lymphocytes was strikingly loose in cases XX XVI *and* XXVII (Plate III 5) and slightly so in case II In 9 cases (I II IX XX XVI XXII XXIII XXX XXVII) 10 % or more of the lymphocytes had nucleoli Particularly in cases where this was common (Case I 38 % XX 42 % XVI 48 %) the nucleoli were large and here and there they were multiple In cases XVI XX XXIII XXX and XXVII about one fourth or more of lymphocytes had a raggy cytoplasm Cells of the type called small lymphoid reticulum cells (Rohr 1960 *see* Plate III 4) could in accordance with what was said above be regarded as present particularly in 7 cases (I II XX XVI XXIII XXX XXVII *in case* XX with 30 % lymphocytes the nuclear structure was blurred by poor staining) The highest concentration of the M components noted in these cases were 1 6 1.2 3 4 2 4 0 7 0 6 and 0 4 g 100 ml

A marked increase of plasma cells was never seen (Table VIII) None of the cases showed a high number of cells representing morphological transitions between plasma cells and lymphocytes Intranuclear inclusions were found in about 4 % of the plasma cells in case XI

The number of mast cells in 10 cases was increased i e 3 or more cells were found in one smear (Table VIII) In case I there were 29 mast cells per 25 plasma cells of which there were 0 4% and in case II there were 31 mast cells per 100 plasma cells, of which there were 0 7 %

In case XIX there were 35 % atypical cells of varying size with a round or oval *sometimes eccentric nucleus with* a fine homogeneous or loose network of chromatin and often with nucleoli and P A S negative inclusions The pale cytoplasm was sometimes diffusely outlined (Plate III 6 7)

In case XXIX there were 46% atypical cells of varying size with a round oval *or* kidney shaped nucleus with fine homogeneous chromatin, sometimes with one or more small nucleoli The pale cytoplasm was diffusely outlined (Plate III 8 9)

Bone marrow puncture was not done in cases XVII XVIII XIX and XXI where histologic examination of a necropsy specimen showed infiltration of cells conceived as plasma cells In case XIX *there was also* an increased number of lymphocytes

COMMENTS

Ever since Waldenström (1944 1948) described the bone *marrow cytology* of macroglobulinaemia (for coloured illustrations see Waldenström 1958 b) infiltration of lymphoid cells and a varying degree of plasmocytosis has been reported as the dominant feature (Creyssel *et al* 1957 Undritz 1957 Imhof 1958 p 53 Kappeler et al 1958)

The average number of lymphocytes in the *normal* bone marrow varies from

Table VIII *Cases with*

Case[1]		Sex Age	R B C (mill)	Bone marrow[2] plasma cells (%)	lympho cytes (%)	mast cells	Albumin	Gamma fraction (g/100 ml)	M component[3] I	II (g/100 ml)	Duration[4] (yr mth)
Living											
I	387	F 75	3 6	< 1	73	+	4 6	0 6	1 6	1 2—1 2	5— 4
II	339	F 86	3 5	2	18	+	4 5	0 7	1 2	0 8—0 9	5— 9
III	460	F 67	4 7	5	20	—	4 8	0 6	0 8	0 8—0 7	4— 7
IV	681	M 50	3 8	2	12	—	2 9	0 6	0 8	0 8—0 0	0— 1
V	999	F 82	3 5	2	18	+	3 8	2 1	0 6	0 6—0 4	1— 0
VI	739	F 53	4 3	1	10	—	4 7	0 6	0 5	0 5—0 3	2— 4
VII	505	F 76	4 8	2	14	—	4 5	1 2	0 4	0 4—0 3	3—11
VIII	959	M 67	4 5	1	10	—	5 4	1 2	0 3	0 2—0 3	1— 2
IX	706	F 50	4 3	< 1	13	—	4 6	0 5	0 3	0 3—0 2	4— 2
X	717	F 71	4 1				3 2	0 5	0 2	0 2—0 0	2— 5
XI	756	F 83	4 6	2	10	—	4 2	0 7	0 2	0 2—0 2	2— 2
XII	775	M 40	3 8	< 1	21	+	5 1	0 7	0 2	0 2—0 2	2— 1
XIII	972	M 22	3 6				3 7	1 4	0 2	0 2—0 0	0— 1
Dead											
XIV	618	M 63	2 8	35*		—	4 0	0 9	6 7		0— 4
XV	24	M 69	2 8	< 1	52	—	2 7	≈ 0 5	5 4	4 0—5 4	1— 3
XVI	27	F 84	2 7	< 1	45	+	2 9	0 7	2 4	1 6—2 4	5—10
XVII	95	M 73	3 4				2 7	0 8	2 3	0 9—2 3	5— 8
XVIII	447	F 87	3 5				3 2	0 6	1 9		0—10
XIX	116	M 85	4 0				3 0	≈ 0 5	1 7		0— 3
XX	36	M 81	4 0	2	30	+	2 1	≈ 0 5	1 6	1 5—1 6	0— 3
XXI	750	F 85	3 8				3 1	1 0	1 3		0— 3
XXII	1256	F 84	4 0	2	17	+	4 4	0 7	0 8		0— 1
XXIII	656	M 79	5 2	< 1	29	—	4 7	0 7	0 7	0 7—0 7	2— 1
XXIV	198	M 59	4 0	4	6	+	4 0	1 8	0 7	0 7—0 4	0— 5
XXV	762	M 78	3 4	1	22	+	4 8	0 8	0 6		0 — 5
XXVI	574	F 80	4 7				3 9	1 1	0 5	0 4—0 5	1 — 7
XXVII	658	M 93	1 7	2	37	+	2 6	0 4	0 4		0— 2

[1] Second column gives the immunoelectrophoretic number for identification
[2] Most abnormal smear
[3] *R B C albumin gamma fraction and M component (I) at the time of highest M component* concentration noted (II) first and last electrophoresis
[4] Interval between detection and last after examination first electrophoresis showing disappearance of the M component or death
* Atypical cells see text

series to series (Segerdahl 1935 p 32, Jacobsen *1941*, Gormsen *1942* p *30*, Fadem & Yalow 1951, Altman 1961 *p 138) It is thus difficult to give any* absolute upper normal limit for the number of lymphocytes In the range 20 — 30 % an increase could however, be suspected A number of lymphocytes of 30 % or more was seen in 5 of the present 20 cases examined and particularly in *those with a relatively* high concentration of M component Case XXVII with 37 % lymphocytes in the bone marrow had however, a discrete component of only 0 4 g/100 ml

The finding of lymphoid reticulum cells is of some diagnostic importance particularly *if the lymphoid*

M-component of type γM

Diagnosis or state of health at time of detection of M component	Diagnosis or state of health at last after examination or necropsy findings
Macroglob. Waldenström	Macroglob. Waldenström
Haemolytic anaemia	Haemolytic anaemia
Respir. tract infection	Mammary cancer
Rheumatoid arthritis	Rheumatoid arthritis
Arterioscler. chron. pyelonephritis	Arterioscler. chron. pyelonephritis
Uterine bleeding	Cardioscler.
Temporal arteritis	Arterioscler.
Coxarthrosis	Healthy (coxarthrosis)
Oligodendroglioma	Oligodendroglioma
Cholecystitis	Uterine cancer
Frequency study senile dementia	Senile dementia
Alcoholism	Alcoholism
Malaria (plasmod. ovale)	Healthy
Macroglob. Waldenström	Rupt. mesenteric aneurysm plasmocytosis
Macroglob. Waldenström	Lymphoid cell infiltr., pulmon. cancer
Macroglob. Waldenström	Lymphoid cell infiltr.
High E.S.R. at routine control	Vesical and colonic cancer plasmocytosis
Femoral neck fract. deterior.	Cardioscler. pyelitis plasmocytosis
Tumour of biliary tract	Papilla Vateri cancer lymphoid cell infiltr.
Pulmon. tumour	Cholangioma
Frequency study cystitis arterioscler. gangr.	Pyelitis pleur. empyema cardiac amyloid
Heart fail. haemolytic anaemia achalasia	Cardiac amyloid achalasia chron. pneumonia
Frequency study healthy	Hepatic and prostatic cancer
Reticulosis (?)	Thrombotic and haemorrh. diathesis
Frequency study arterioscler. deterior.	Encephalomal. pneumonia intracran. neurinoma
Stroke	Slight incr. of bone marrow plasma cells and reticulum cell
Gastric cancer	Gastric cancer

infiltration is not so marked. To decide whether a cell should be regarded as a mature lymphocyte or lymphoid reticulum cell may be difficult (Kappeler et al. 1958). Therefore only when numerous cells with characteristics of the last mentioned cell were found was the number of lymphoid reticulum cells regarded as increased.

Unlike what has been seen in many other series no plasmocytosis was found except in the 4 cases examined histologically. Intranuclear inclusions (Lelbach 1957, Dutcher & Fahey 1959) in *lymphocytes* and plasma cells were seldom seen.

A slight to clearly increased *number of* mast cells (Tischendorf & Hartmann 1950, Undritz 1957, *Kappeler et al.* 1958) was seen in half of the cases and this together with a more or less clearly increased number of lymphocytes led the examiner's thoughts to macroglobulinaemia Waldenström in 8 cases (I, II, V, XII, XXI, XX, XXV, XXVII).

Markedly atypical cells of the type described by Lennert (1955), Oettgen & *Quitmann* (1956) and Lelbach (1957) and corresponding to Zollinger's (1958) fourth group were seen in case XIV. Some of these cells had plasmacellular

features This, together with the occurrence of nucleoli, suggests that they produced the M component Also in case XXIV, which was difficult to judge clinically and histologically, the bone marrow and the blood contained atypical cells The relation between these cells and the occurrence of an M component is obscure

ELECTROPHORETIC FINDINGS

RESULTS

The concentration of serum albumin (Fig 22, Table VIII) was subnormal in 15 cases (< 4 2 g/100 ml) Eleven of these 15 patients had an increased concentration of the alpha$_2$ fraction Of the 2 with hypoalbuminaemia, normal concentration of the alpha$_2$ fraction and an M component concentration of 0 4 g/100 ml or less, one had malaria (XIII) and one (XXVII) bleeding gastric cancer

The concentration of the gamma fraction was subnormal (< 0 8 g/100 ml) in 14 of the 24 cases in which the

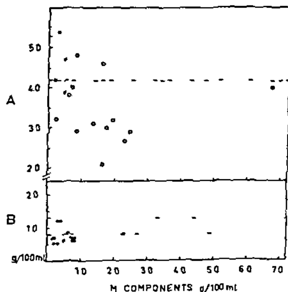


Fig 22 Concentration of albumin (A blank symbols increased concentration of alpha fraction) concentration of gamma fractions (B) in 24 cases in which it could be estimated with acceptable accuracy and concentration of M component Broken line normal range

fraction could be estimated with acceptable accuracy (Fig 22) and in 10 of 17 cases with M components in a concentration below 1 0 g/100 ml In 3 cases the concentration of the gamma fraction exceeded the normal range and of these patient No V had chronic pyelonephritis No XIII had malaria and No XXIV a clinical picture difficult to interpret

The highest concentrations of the M component noted were 6 7, 5 3 and 2 4 g/100 ml In 10 cases the concentration exceeded 1 0 and in 10 it did not exceed 0 5 g/100 ml (Table VIII)

An increase of the concentration of the M component was noted during various periods in cases XV, XVI and XVII As a rule, however, the concentration was low and constant (Fig 23) In cases IV, X and XIII the component disappeared (Chapter VI)

In 3 cases the beginning of the synthesis of the M component could be approximately dated In case VII, in which the patient was ill with temporal arteritis electrophoresis in May and August 1960 showed no M component, but in April 1961, by which time the symptoms of arteritis had disappeared in association with phenylbutazone therapy an M component was demonstrated The sum of the gamma fraction and the M component was then equal to that of the gamma fraction before the discovery of the component —Patient XXIII had no M component in 1957, when a gastric cancer was excised The component (0 7 g/100 ml) was discovered in a frequency study in October 1961 and was of the same concentration in April 1963 Seven months later he died from primary liver cancer —In case XXVI there was diffuse hypergammaglobulinaemia (1 9 g/100 ml) in association with pneumonia in February 1959 The M component (0 3 g/100 ml) did appear in October 1961 when she was in hospital because of hemiplegia The gamma fraction was then 1 7 g/100 ml

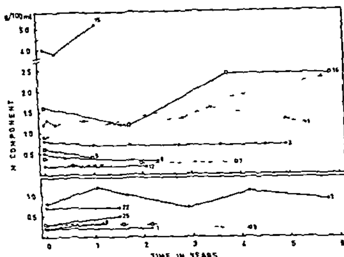


Fig 23 Course of concentration of γM-component in 15 ca es followed up without cytostatic therapy For clarity 2 fields and broken lines are u ed Ca e numbers given in Arabic numerals

A light chain component was *found* in the urine in case XX In cases XXII XXIII and XXXII proteinuria was not sati factorily excluded and in case XXII electrophoresis of the urine was not performed in spite of proteinuria

COMMENTS

Hypoalbuminaemia is common in macroglobulinaemia Waldenström (Imhof 1958 p 90) In the Malmö series about half of the patients had a low albumin concentration In many however the *concentration* of the alpha fraction was increa ed so condition other than macroglobulinaemia may have caused or contributed to the hypoalbuminaemia (see Wuhrmann & Märki *1963* p *282*)

Kappeler et al (1958) observed an increa ed tendency to infection in some ca es of macroglobulinaemia Vargues et al (1961) succeeded in demonstrating that the bactericidal property of the serum is decrea ed in macroglobulinaemia Fahey et al (1963) examined 18 ca es of macroglobulinaemia Waldenström *The* patients had a greater *tendency to infections than* the general hospital population and in immunisation studies the antibody synthesis was found to be decreased In more than 50 % of the ca*ses in the* Malmö *series the* concentration of the gamma fraction was subnormal but in only a few cases was the concentration decreased to the level ($\leq$ 0 5 g/100 ml) which is usually noted in the Antibody deficiency syndrome (Barandun et al 1959)

On analysis of some series of macroglobulinaemia Waldenström (Mandema 1956, Creyssel et al *1957 Imhof 1958* Kappeler et al 1958 Streiff 1959 Martin 1960 Olmer et al 1960) the results of electrophoresis given in 55 cases permit estimation of the concentration of the M component which rarely if ever might have been below 1 0 g/100 ml In contrast hereto in the Malmö series the concentration *in 17 of 27 ca es* was below 1 0 and in 10 it was 0.5 g/*100 ml* or less A concentration of *more than* 1 5 g/100 ml was found mainly in cases where the macroglobulinaemia was *con*ceived as the main disea e and in some

features This, together with the occurrence of nucleoli, suggests that they produced the M-component Also in case XXIV, which was difficult to judge clinically and histologically, the bone marrow and the blood contained atypical cells The relation between these cells and the occurrence of an M component is obscure

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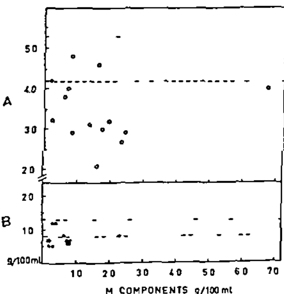


Fig 22 Concentration of albumin (A blank symbol increased concentration of alpha$_2$ fraction) concentration of gamma fractions (B) in 24 cases in which it could be estimated with acceptable accuracy and concentration of M components Broken lines normal range

case I An unexplainable high E S R was recorded 7 years before the woman sought advice for fatigue and anorexia when anaemia and an M component were demonstrated For 3 years after the last mentioned finding the woman was repeatedly admitted to hospital, mainly because of fatigue but she always improved without any special treatment During this time the lymphocyte infiltration in the bone marrow seemed to increase During the following 2 years she did fairly well she did not require hospital care and the concentration of the M component remained largely unchanged The bone marrow lymphocytosis however progressed as did the anaemia

The main *cause of death* was macroglobulinaemia Waldenström in case XX and probably also in case XXI — In case XIX the serious course of macroglobulinaemia was cut short by rupture of an arterial aneurysm — The cause of death in case XXIII was obscure The patient deteriorated rapidly during an initially successful convalescence after a fracture The M component (1 9 g/100 ml) was discovered on electrophoresis performed because of an increased E S R (93 mm/1 hr) in association with a fracture 10 months before death Sternal puncture was not done and electrophoretic examination was not repeated Necropsy failed to provide any explanation for the final clinical picture but revealed bone marrow plasmocytosis

The observation period was on the average 32 months in the living group and 16 months in the *group* who had died In 3 cases (p 64) electrophoresis had been done before the appearance of the M component and in the other cases nothing is known about the true persistence of the component In cases I XX XXI and XVII the E S R had been recorded before the finding of an M component (Fig 24) In case XX the

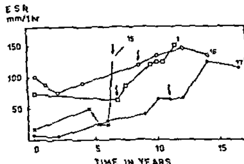


Fig 24 Course of E S R in 4 cases with γM component Arrows show finding of M component Case numbers given in Arabic numerals

E S R suddenly rose after some values of about 20 mm/1 hr had been noted At the same time specific symptoms appeared and the patient died one year later

Patient II probably had her first attack of haemolytic icterus in 1950 In 1959 cold agglutinins were found as well as an M component, whose concentration was largely unchanged in 1965 (0 9 g/100 ml) Patient XXII had had symptoms that could be related to cold agglutininaemia for 10 years before the discovery of such agglutinins and an M component

COMMENTS

In this investigation the term macroglobulinaemia Waldenström was used only for 4 cases in which a more or less progressive clinical picture could be related only to the finding of an increased activity of the macroglobulin synthesizing cells as reflected in a serum M component and a more or less typical cytological picture of the bone marrow Objections may be raised against such a procedure but here it was used simply to distinguish the cases corresponding to those which are reported in the literature as macroglobulinaemia Waldenström and in which the clinical features could not be explained by other diseases

patients with cancer Though the number of cases followed for a long time was small, the same tendency could be discerned as in cases with M components of type γG or γA, i e some cases were stationary with usually low concentration of M component and unrelated or no symptoms while others show an increasing concentration of the component and develop a serious clinical picture (Waldenström 1965)

In 3 cases electrophoresis had been performed before the appearance of the M component Of interest is particularly case VII, where an M component of 0 4 g/100 ml appeared in the course of half a year and later (4 years) remained largely constant Similar cases have been described in chapter III

CLINICAL PICTURE AND COURSE

RESULTS

Cases I, XIV, XV and XVI showed a *clinical picture* for which no explanation could be found except the abnormal activity of the γM synthesising cells These cases were classified as macroglobulinaemia Waldenström

Enlarged lymph nodes were never palpated but at necropsy they were seen with lymphocytic infiltration in cases XV and XVI Enlargement of the spleen was palpated in case XXIV, and in case XIII, in which the patient had malaria Enlargement of the liver was palpated in cases XIX and XXIII and was due to tumour growth

Mucosal bleeding (gingival bleeding but also parodontosis) was seen only in case XV, in which the patient finally developed purpura the number of thrombocytes was normal Purpura was also found in case XXIV with a thrombocyte count of about 70,000/mm³

Neurologic symptoms and signs dominated the picture in case XIV with a short history of dizziness, vomiting and headache He was markedly somnolent and 2 weeks after admission he died from shock because of a ruptured aneurysm The only explanation for the symptoms for which he was admitted was macroglobulinaemia (6 7 g/100 ml) In case XV the patient was troubled by pain and numbness of the limbs, symptoms which temporarily regressed in association with plasmapheresis

Changes in the ocular fundi in the form of haemorrhage and congested, beaded veins were seen in case XIV In case XV ophthalmoscopy showed a normal appearance but was performed only on the occasion when the M component (3 9 g/100 ml) was discovered In cases I, XVI and XXIV nothing of interest was found Otherwise only the living were examined and were found to have normal fundi In case XVI there was a granular flow in the conjunctival vessels

Weakness and loss of bodyweight were common features of the clinical picture in the patients who had died, but many of the patients had co existing diseases such as malignant tumours or chronic infections In 3 members of the living group the M component disappeared, and also in cases III, V, VI, VII, VIII, IX and XI the clinical pictures could not be related to the occurrence of a discrete component

The course of the disease, as judged from the clinical picture, E S R and electrophoresis, progressed rapidly in case XV (Figs 23 and 24) —Also in case XIV symptoms rapidly developed which could only be ascribed to macroglobulinaemia —In contrast, the course in case XVI was slow as, in case XVII An M component was discovered in the serum from No XVII 5 years after the first recording of an increase of the E S R and 3½ years before supervention of symptoms of a tumour from which the patient died 2 years later The M component had by then increased (0 9—2 3 g/100 ml) —A very slow progressive course was also seen in

case I An unexplainable high E S R was recorded 7 years before the woman sought advice for fatigue and anorexia when anaemia and an M component were demonstrated For 3 years after the last mentioned finding the woman was repeatedly admitted to hospital mainly because of fatigue but she always improved without any special treatment During this time the lymphocyte in filtration in the bone marrow seemed to increase During the following 2 years she did fairly well she did not require hospital care and the concentration of the M component remained largely un changed The bone marrow lympho cytosis however progressed as did the anaemia

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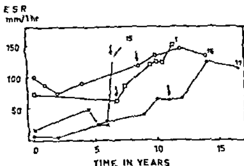


Fig 21 Course of E S R in 4 cases with M component Arrows show finding of M-compo nent Case numbers given in Arabic numerals

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COMMENTS

In this investigation the term macro globulinaemia Waldenström was used only for 4 cases in which a more or less progressive clinical picture could be related only to the finding of an in creased activity of the macroglobulin synthesizing cells as reflected in a serum M component and a more or less typical cytological picture of the bone marrow *Objections may be raised against such a procedure but here it was used simply to distinguish the cases corresponding to those* which are reported in the literature as macroglobulinaemia Wal denström and in which the clinical features could not be explained by other diseases

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Macroglobulinaemia Waldenstrom differs from myelomatosis *int r alia* by its more chronic course Observation times of 8 to 16 years have been de cribed by e g Bichel et al (1950) Mackay et al (1956) Fiere (1957) Martin (1960) Olmer et al (1960) and Riva (1964) The observation time in the Malmo series do not say how long γM components can exist The components were usually detected at examinations because of conditions which presumably had nothing to do with macroglobulinaemia and only in a few cases had electrophoresis been done before the detection of the M component Moreover the cause of death in many cases was some disease other than macroglobulinaemia Waldenstrom In so far as a high E S R and signs of cold agglutininaemia can be accepted as evidence of an M component such a component was sometimes present long before it was demonstrated electrophoretically an observation reported already by Schulten & Kanzow (1956)

CO EXISTING DISEASES

RESULTS

Neoplasms of varying type and localisation occurred in 9 cases in the group of patients who had died and in 3 of the living Case XXV had an intracranial neurinoma which was an incidental finding at necropsy In case XXIV the picture was difficult to interpret There were among other things atypical cells in the bone marrow cells which might represent a malignant growth (reticulosis? p 61)

The patient in case IX had an oligodendroglioma The tumour had caused single epileptic attacks and slight aphasia half a year before the discovery of the M component and was operated upon about 5 years later In the other 9 cases

the patients had cancer The time (year) of onset of symptoms of tumour before or after detection of M component was

Case	Before	After
IX	½	
XIX	½	
XX	½	
XXVII	[illegible]	
XXIII	[illegible]	2
XVI	18	
XV		1½
III		2
X		2
XVII		3½

In case X a discrete component was found at examination because of an attack of cholecystitis By the time of the first after examination 2½ years later the component had disappeared but the patient was then found to have uterine cancer —Case XV had a pulmonary cancer discovered at routine histological examination at necropsy —In case XVII the M component was discovered at investigation because of increasing F S R 3½ years before the onset of symptoms of a tumour which resulted in death after a further 2 years

The patient in case XXIII was operated upon because of an adenocarcinoma polyp of the stomach in 1957 when electrophoresis had shown no discrete component The tumour had bled for about one year An M component was demonstrated in a frequency study of M components in the autumn of 1961 In April 1962 the patient was investigated further because of this finding and then the only abnormality found was a serum bilirubin in a concentration *bordering the upper normal limit (1.2 mg 100 ml)* and decreased prothrombin content At after-examination in April 1963 he was in a *good condition* his bodyweight and blood values were unchanged as was the electrophoretic pattern Three months later he *began to* deteriorate and a tumour was palpated below the right costal arch *At necropsy* 1 month later primary liver cancer was diagnosed There were no signs of gastric cancer

Of *other diseases* in the material mention might be made of haemolytic anaemia (cases II and XXII) chronic infections of the urinary and respiratory tract (V XVIII XXI XXII) Acute infections such as cholecystitis (X) and malaria (XIII) and various conditions such as subacute rheumatoid arthritis (IV) arteriosclerosis (VI VII, XI XXV XXVI) coxarthrosis (VIII) and alcoholism (XII)

COMMENTS

The possibility of a relation between macroglobulinaemia Waldenström and cancer was discussed as early as 1953 by Schaub Kappeler et al (1958) traced 18 cases of cancer in the literature out of 146 with macroglobulinaemia Nine of the 27 subjects in the present material had or had had cancer As in group I (p 56) it is difficult to draw any conclusions about a possible relation between tumour and macroglobulinaemia from the frequency of cancer in this *series* It is also difficult to draw any conclusions from the chronological *order of the* discovery of tumour and M component Sometimes the tumour was discovered first sometimes the discrete component and how long these 2 *had actually* existed is impossible to *decide*

Of the patients with other co-existing diseases *the one with malaria is perhaps* most interesting The *finding of an M-component in a young person is* unique *(Axelsson et al 1966) and together with the fact that the component disappeared in association with* successful treatment strongly argues for the assumption of a relation between the disease and the electrophoretic finding Secondary macroglobulinaemia is sometimes seen in *inter alia* certain infectious *diseases but* is then a part of a generally increased immunoglobulin concentration (Waldenström 1958 a Jahnke & Scholtan 1960 p 84) It is however of

Waldenstrom (1965) and Seligmann (1966) stressed that the distinction between malignant (progressive) cases and benign (stationary) cases is more diffuse in a group of cases with γM-components than in one with γG- and γA components, and this is also clear from this extended series from Malmo For example, in some of the cases with M *components in moderate or low* concentration the appearance of the bone marrow was compatible with the diagnosis of macroglobulinaemia Waldenstrom, while the clinical picture otherwise *showed nothing in support of such* a diagnosis Of interest in this respect is case XII, where the clinical diagnosis was chronic alcoholism For 2 years the M component remained unchanged (0 2 g/100 ml), but an unexplained anaemia and atypical bone marrow findings were noted It is true that the bone marrow lymphocytosis (21%) need not be regarded as abnormal and the lymphocytes were morphologically normal, but mastocytosis was evident This could not be explained by any of the other *conditions except macroglobulinaemia* Waldenstrom, where mastocytosis is observed, above all bone marrow lesion ("aplastic anaemia") primary or in association with malignant neoplasms or severe infections (Bremy 1950, Fadem 1951, Undritz 1957) One might, of course, wonder whether case XII is an *example of an early macroglobulinaemia* Waldenstrom Cases with but few or no symptoms have been published by *inter alia* Kappeler et al (1958), Francke & Robert (1960), Riva (1964) and Seligmann et al (1965) Cases with M-components in such a low concentration as in cases XII and XXVII (0 2, 0 4 g/100 ml) but nevertheless with a cytological picture suggesting macro globulinaemia Waldenstrom have, however, seldom been described (Axelsson et al 1966) The present material differs from others (e g Kappeler et al 1958, Bothier et al 1960) also in that it *contains* a number of cases with neither clinical nor cytological counterpart to the γM component

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CHAPTER V

M-COMPONENTS IN LEUKAEMIA IN LYMPHOSARCOMA AND IN RETICULUM CELL SARCOMA

M components have relatively often been found in the sera from patients with chronic lymphatic leukaemia lymphosarcoma and reticulum cell sarcoma Spengler et al (1961) published 6 cases with M components and chronic lymphatic leukaemia They also gave a number of references to authors who had made the same observation To the cases which were examined with immunoelectrophoresis and/or ultracentrifugation of the serum others can now be added so that the number of case of lymphatic leukaemia (sometimes conceived as transitional forms of macroglobulinaemia Waldenström) with well defined M component will be 23 (Mackay et al 1957 Imhof 1958 p 54 Mielke 1958 Braun teiner & Sailer 1960 Heremans 1960 p 270 Rebuck 1960 Spengler et al 1961 Carrod 1963 Creyssel et al 1964 Hallen 1964 Seligmann & Burtin 1964 Axelson et al 1966 van Furth et al 1966 Camble & Cutting 1966 Krauss & Sokal 1966) The M components in these cases were γG in 6 γA in one and γM in 16

Märki & Siegenthaler (1964) described a case conceived as *paramyeloblastic* leukaemia with a γM component in the serum and Bence Jones protein in the urine *M components* have also been observed in the *serum from* patients with chronic and with acute *myeloid* leukaemia osteomyelosclerosis and polycythaemia vera (Heremans 1960 p 270 Klima et al 1962 Kanzow et al 1964 Videbæk & Drivsholm 1964 Creyssel 1965)

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CHRONIC LYMPHATIC LEUKAEMIA

To find out *inter alia* whether M components are abnormally common in chronic lymphatic leukaemia an unselected series of this disease was studied It was not possible to collect an unselected series of patients with lymphosarcoma or with reticulum cell sarcoma

MATERIAL

The material consisted of 40 men and 19 women diagnosed from 1954 to the end of June 1965 All were from Malmö and represented practically all cases of this disease seen at hospital during the period in question One case with coexisting myelomatosis was excluded because the M-component in that case was ascribed mainly to plasma cell proliferation (Waldenström 1962 case A T)

RESULTS

The age distribution and the number of lymphocytes are given in Table IX

interest to note that Sonnet & Michaux (1964) saw discrete components remarkably often in sera from Bantu negroes who often had polyclonal hypergamma globulinaemia The components which were generally of type γG could, according to the authors, possible be conceived as part of an immunological response to the parasitic and infectious diseases, which are common in those regions

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Table X Data on 15 cases of l ka m a o arcoma (lympl o n oma r ul n ll a con a)

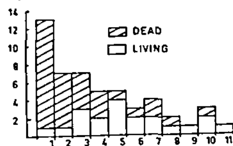
Case		Sex Age	Enlarged lymph nodes	Enlarged spleen	Enlarged liver	Lymphocytes[2] a (10^3/mm^3)	Lymphocytes[2] b (10^3/mm^3)	M component (g/100 ml)		Duration[3] (yr)	Diagnosis
1†	581	M 63	+	++	++	122	0.5	0.1	γM	1–10	Chronic lymphatic leukaemia
2†	654	M 80	++	+	+	12	1?	0.4	γM	1–4	Chronic lymphatic leukaemia
3†	758	M 70	++	++	+	275	16	0.1	Light chain	3–10	Chronic lymphatic leukaemia
4†	319	M 73		+		10	4	2.3	γG	2–0	Chronic lymphatic leukaemia
5	59	M 42				195	125	0.2	γG	19–2	Chronic lymphatic leukaemia
6†	1096	M 53	+	+		21?	??	0.2	γM	4–6	Chronic lymphatic leukaemia
7	961	F 48	++	+		34	14	?1	γM	2–2	Chronic lymphatic leukaemia
8†	?W	M 62	++			10?	35	0.4	?	4–0	Chronic lymphatic leukaemia
9†	26	F 69	—	++	++			1.0	γM	2–9	Blast cell leukaemia
10†	37	M 51	—		—			0.8	γM	5–10	Lymphosarcoma
11†	372	F 90			—			0.6	γM	0–2	Lymphosarcoma—reticulum cell sarcoma
12†	110	M 60	++	—	++			1.3	γG	0–7	Lymphosarcoma—reticulum cell sarcoma
13†	258	M 88	—		—			0.6	γM	0–4	Reticulum cell sarcoma
14†	101	F 62	+	++	++			0.3	γG	0–1	Reticulum cell sarcoma
15†	719	M 67	+		—			0.3	γG γA	0–3	Reticulum cell sarcoma

[1] Second column gives ... immunoelectrophoretic ... nl er or n nls
[2] Il gl est n l er rec r le l (a) n n l er at f l ng of M co npo ent (l)
[3] I ervol l etween l ag s a l len l or c l of tl a n es gnt o

Table IX *Age at last electrophoresis before cytostatic or corticosteroid treatment or at detection of an M component, and number of lymphocytes in an unselected series of 59 cases of chronic lymphatic leukaemia Bracketed figures denote males*

Age	Number of patients total	with M components
40—49	1 (0)	1
50—59	7 (5)	2
60—69	15 (11)	2
70—79	29 (19)	2
80—99	7 (5)	1
Lymphocytes (1000)		
5—9	3	0
10—24	6	1
25—49	15	2
50—99	12	0
≥100	23	5

The age used was that at the time of detection of an M component or at the last electrophoresis before any treatment with corticosteroids or cytostatics was instituted The interval between diagnosis and the last mentioned electrophoresis was half a year or less in 29 cases and ranged from ½—2 years in 4, from more than 2 to 4 years in 10 and from more than 4 to 9 years in 8 cases (excluding the 8 with M component) A representative value for the number of lymphocytes was noted and in cases with leukocyte values below 100,000/mm³ the highest value found was selected The smallest number wa 6,500 (W B C 11,300) The observation time at the end of June 1965 and the number of patients living at that time are given in Fig 25 Enlargement of the lymph nodes was noted in 50 patients and palpable enlargement of the spleen in 41



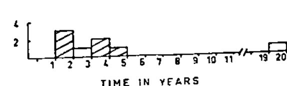


Fig 25 Interval between diagnosis and death or end of investigation period in 59 cases of chronic lymphatic leukaemia The 8 cases with serum M component at bottom

Of the 59 patients, 8 proved to have serum M components The following remarks refer to these 8 cases

On the basis of the frequency of M components in the different age groups of either sex (Axelsson et al 1966) the expected number of cases with M components in the leukaemia series was calculated at 1 4 of 59 The frequency found was significantly higher than the expected frequency (8 > 1 4 + 2 5 × √1 4)

Seven of the 8 cases were men The age distribution of the cases largely resembled that of the total leukaemia series (Table IX) In table IX the ages given are those at the finding of the M component and in table X those at the diagnosis

Enlarged lymph nodes were found in 6 cases and splenomegaly in equally many The liver was enlarged in 3 cases (Table X)

Anaemia (R B C ≤ 3 5 mill) was noted throughout the disease or during parts of the disease in all cases except No 5 where the R B C was still normal at the last examination, after 19 years In 5 cases (3 4, 6, 7, 8) anaemia was so severe that blood transfusions were indicated In 3 cases (4, 7, 8) there were

only hypogammaglobulinaemia (0 6 g/100 ml), the M component was found at the next examination (October 1964) 9 months before death

The duration of the disease is given in Table X and Fig 25 On June 30, 1965 only cases 5 and 7 were alive and case 7 died in November that year

The clinical course showed nothing remarkable in cases 2 (deterioration + pneumonia) 3 (anaemia + pneumonia), 6 (anaemia + thrombocytopenia with bleeding tendency + pneumonia) and 8 (final anaemia + severe tendency to infection) In cases 2, 7 and 8 necropsy showed a picture compatible with reticulum cell sarcoma respectively lymphosarcoma—reticulum cell sarcoma

Case 1 responded well haematologically to chlorambucil therapy but the patient deteriorated successively, and was troubled by vomiting abdominal pain constipation and diarrhoea Finally a number of lumps were palpated in the abdomen and were conceived as lymph nodes and a discrete component was demonstrated Necropsy was not allowed

Case 4, whose case history has been discussed previously (Waldenström 1962 case G N) was in some respects atypical The peripheral blood picture was mostly aleukaemic there was severe thrombocytopenia and the histological changes in the spleen and lymph nodes were insignificant The marked lymphocytic dominance of the bone marrow and particularly the peripheral blood picture at the end of the course were such that he was assigned to the group chronic lymphatic leukaemia

Case 5 was an example of a benign course After the diagnosis had been established incidentally the course for 19 years was slowly progressive as far as the number of white blood cells is concerned (18 000 in 1946 56,000 in 1956 136 000 in 1961 and 124 000/mm^3 in 1965) The patient's general condition was good and the M component which was discovered at follow up after 15 years, remained unchanged

The course in case 7 was characterised by severe progressive symptoms An M component in the serum was observed early in the course as was a light chain component in the urine The concentration of the M component increased markedly during a period when lymphocytosis regressed in association with cytostatic therapy and the clinical picture first improved but afterwards became worse Finally, hypercalcaemia and skeletal lesions were observed, which at necropsy were found to correspond locally to massive lymphocyte infiltrates in the periosteum Histologically, there were signs of transition into lymphosarcoma reticulum cell sarcoma

COMMENTS

The questions that arise are above all Is there any relation between the disease and the occurrence of an M component? Which cells in these cases synthesise the protein corresponding to the M component? Do cases with discrete components in the *serum* differ in any way—particularly prognostically—from other cases of *lymphatic* leukaemia? Should the patients with γM components be regarded as having macroglobulinaemia Waldenström with increased number of lymphoid cells in the blood?

As to the first question in the present material the frequency of M components in the serum was significantly higher than expected Of the 8 cases 4 had γM *components* In one case the component was not typed Irrespective of this case at least half had a discrete component of type γM which is an overrepresentation (P = 1 4%) since only 7 of 64 persons with M components found in a population study (Axelsson et al 1966) had components of type γM Together with the literature reports (p 71) it would thus appear that the

signs of haemolysis, above all, an increased carboxyhaemoglobin Coomb's test was positive only in case 8 (1 128) The occurrence of cold agglutinins could be excluded in all cases, but only 4 were examined in this respect while an M component was demonstrable

All showed an increased number of lymphocytes in the blood on some occasion, though in case 4 this increase was moderate (at most 10,000/mm^3) The distribution of the cases in this respect was largely the same as that in the total leukaemia series (Table IX)

Bone marrow puncture was done in 5 cases (2, 4, 5, 7, 8) Lymphocytes of varying degree of maturity dominated in all The smallest number of lymphocytes was seen in case 5 (1961 57 % and 1965 74 %) The lymphocytes were usually of normal morphologic appearance The occurrence of nucleoli and the number of large cells with a fine chromatin structure (lymphoblasts, Plate II 4) was

Case	Nucleoli	Lymphoblasts
2	29 %	10 %
4	3	3
5	13	3
7	14	4
8	35	5

Most of the lymphoblasts contained large nucleoli and there was a gradual transition between these and mature lymphocytes The number of plasma cells was invariably low and at most single cells were seen despite a thorough search In 3 cases an increased number of mast cells were found Below are given under I, case number and immunological type, under II, the number of plasma cells per 1,000 nucleated cells under III the number of mast cells per number of plasma cells found after a thorough search

I	II	III
4 γG	2	10/5
5 γG	1	6/50
8 ?	0	7/10

The concentration of the M components did not exceed 0 4 g/100 ml with the exception of 2 cases, but one of the components was of the light chain type (Table X) The concentration of the component in case 4 increased from 0 7 to 2 3 and in case 7 from 0 2 to 2 1 g/100 ml

The immunological type of the components was γG in 2 cases, γM in 4 and light chain in one (Table X) In case 8 the type of the component was not determined

In cases 2, 3 and 7 paper electrophoresis of the urine revealed a light chain component which was excreted in a small amount except in case 7 (1–2 g/24 hrs) Case 5 had no proteinuria and in the other cases proteinuria was not satisfactorily excluded

An M component was found at the first electrophoretic analysis performed at the clinical onset of the disease in 3 cases (4, 7, 8) — In case 2 the patient himself had observed enlargement of the lymph nodes for 2 years before he had sought medical advice, and a discrete component was found in the serum — In case 5, where the diagnosis was established in 1946, an M component was observed at the first electrophoretic examination, which was performed at routine control in 1961 — In case 1 a component appeared after 2 years' disease the concentration of the gamma fraction was normal (≥ 0 9 g/100 ml) in January and August 1960, but in September 1961, after prednisone and chlorambucil treatment for one year, it was 0 4 g/100 ml, as it was also in November 1961, when the M component appeared, 2 weeks before the patient died — In case 3 a component was seen one month before death (January 1963), electrophoresis in Jan 1962 and twice in 1959 when the diagnosis was established showed only hypogammaglobulinaemia (≤ 0 6 g/100 ml) — In case 6 the first examination (November 1960) showed

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abnormally high frequency of M-components was due to an increased tendency of the patients to develop, above all, components of type γM

It was much more difficult to decide which cells produced the M components Plasma cells, which are the cells principally associated with production of immunoglobulins (p 26), were not increased in number in the bone marrow smears But neither in group I was bone marrow plasmocytosis regularly found (Fig 4, p 22) and plasma cells also occur in sites other than the bone marrow and puncture of *e g* lymph nodes or spleen had not been done In group I practically all of the patients with a component of more than 1 5 g/100 ml had an increased number of plasma cells in the bone marrow This was not so in the patients No 4 (γG) and No 7 (γM) with leukaemia In these patients, as in the others, also the absolute number of plasma cells appeared to be low, which was rare in group I This suggests the possibility that cells other than plasma cells synthesise the M component protein and one then wonders whether the protein might have derived from the lymphocytes Various observations and examinations argues for the lymphatic cells having some connection with—possibly synthesising—immunoglobulins and then perhaps, above all, those of macroglobulin type For example, in macroglobulinaemia Waldenstrom the cytologic picture is most often dominated by lymphoid reticulum cells and, judging from occurrence of ergastoplasm they synthesise protein (Braunsteiner et al 1956) In addition, Abrams et al (1949) succeeding in extracting macroglobulin from lymphosarcomatous lymph nodes In immunisation experiments with typhus vaccine during the neonatal period when lymph nodes do not yet contain plasma cells but small and large lymphocytes and reticulum cells, Smith (1960) found that H antibodies were not of the usual type 7 S but of type 19 S Zucker-Franklin et al (1962) found that ^{14}C labelled lysine was incorporated in 19 S globulin, which could be eluted from the lymph nodes from patients with macroglobulinaemia Waldenström when the lymph nodes were incubated with the amino acid in question The authors, who used an antibody fluorescence technique, also demonstrated macroglobulin in the predominant cells in the lymph nodes, namely medium sized and large lymphocytes and lymphoid reticulum cells Macroglobulin has been demonstrated in lymphoid cells with the last mentioned method by several authors (Curtain & O'Dea 1959, Dutcher & Fahey 1960, Burtin 1961, Curtain 1961, Kritzman et al 1961, Solomon et al 1963) Macroglobulin has been demonstrated with this method also in plasma cells (Mellors et al 1961, Burtin & Buffe 1962, Mellors & Korngold 1963, Solomon et al 1963, Chiappino & Pernis 1964), but in small lymphocytes Zucker Franklin et al (1962) and Mellors & Korngold (1963) could not demonstrate such protein The last mentioned cells have, however, proved morphologically labile and under certain circumstances they develop into forms resembling the cells seen in the germinal centres of the lymph nodes, which cells contain immunoglobulin (Elves 1965) In addition Gamble & Cutting (1966) recently published a case of lymphatic leukaemia with a γG component in the serum By immunofluorescence technique large, medium sized as well as small lymphocytes were found to contain the γG component protein

Thus it did not seem improbable that the M components in the present cases were synthesised by lymphatic cells This assumption is supported to some degree by the fact that particularly components of type γM were remarkably common

The 8 cases with M component did

not differ substantially from the rest of the material concerning age and sex distribution hyperplasia of the lymph nodes and spleen and degree of lympho cytosis Spengler et al (1961) found no prognostic difference between a group of ca es with serum M components and other cases with chronic lymphatic leukaemia If we exclude from the present series 3 patients who presumedly died from other di eases the median survival time on June 30 1965 was 3 years both in the group without and in that with M components The last mentioned group was however too small to permit any valid conclusion concerning the prognostic significance of such a component in lymphatic leukaemia The cour es of the cases varied widely from benign in case 5 to a more fulminant course in case 4 and between these extremes *e g* cases 2 and 7 with an initially benign cour e which afterwards became malignant As in other cases (Chapter III and IV) an increa e of the M component concentra tion seemed to be an unfavourable sign The components were mostly of low concentration but did not always appear until late in the cour e when there was not time to demonstrate an increase if any of the concentration or serum electrophoresis was not repeated before the patient died

Whether the patients with a γM component should be classified under the heading macroglobulinaemia Wal denstrom with lymphocytosis or lymph atic leukaemia with γM component is largely a question of definition All had peripheral lymphocytosis and may so far be regarded as instances of lymphatic leukaemia The patients did not show the clinical picture of macroglobulin aemia Waldenström but the concentra tion of the M components was not high enough either to expect the development of a hypervi cosity syndrome (Fahey 1963) Lymphoid reticulum cells mast cells or an increased number of plasma cells or intermediate forms between such cells and lymphoid cells were not seen in the bone marrow It would therefore appear justified to classify these cases as chronic lymphatic leukaemia with γM component

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Case 9 was conceived clinically as blast cell leukaemia but the picture seen at necropsy was difficult to judge In the other 6 ca es there was sarcoma some times dominated by lymphoid cells, sometimes by reticulum cells

The patients age and sex *is given in* Table X which al o includes the inci dence of enlargement of lymph nodes spleen and liver

Anaemia (R B C male < 4 0 female < 3 6 mill) was noted in all cases

The white blood picture in cases 11–15 need no comments In case 9 a varying number of atypical cells were seen in the blood smear (358 000/mm³ at first admi sion) These cells were of varying size with scanty pale cyto plasm structureless or fine chromatin and sometimes one or several nucleoli in a round or lobulated nucleus (Plate II 7) —*In case 10* about 250 cells/mm³ with deep blue cytoplasm were seen the nucleus had a coarse loose chromatin and sometimes nucleoli (Plate III 2, 3)

Sternal puncture was done *in all cases* except in Nos 11 and 13 At 90 % the cells *in smears from case 9* corre ponded to those in the blood though the nuclear *structure* appeared more flocculant (Plate II 8) —*In case 10* five per cent of the cells had oval or irregularly *shaped* nuclei with a coarse and loose chromatin and sometimes nucleoli (Plate III 1) the cells corresponded to those in the blood – In case 12 sixty two per cent of the cells were lymphocytes and lymphoblasts —In case 14 erythro poiesis and leucopoiesis were markedly

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The concentration of the M component was 1 0 g/100 ml or more in only 2 cases (Table X) The component was seen at the first electrophoretic examination except in case 9, in which this examination was not technically successful (1953) Increase in the concentration was noted in case 10 (0 3 to 0 8 g/100 ml in 4 years) and in case 12 (0 4 to 1 0 g/100 ml in 6 months)

The immunological type of the component was γM in 4 cases (Table X) A light chain component was found in the urine in case 9 and was suspected in case 15 In case 11 Bence Jones test was negative In cases 10 and 13 there was no proteinuria, which was not satisfactorily excluded in case 14 The urine was not examined electrophoretically in case 12 despite proteinuria

The course of the disease was usually short (Table X) and always fatal In cases 11, 12, 13 and 14 the course was dominated by deterioration and fever and in case 13 also by repeated pneumonia

In case 9, which has been discussed previously by Waldenström (1962, case G A), the picture was initially that of leukaemia The dominating cells were conceived as blast cells probably from the myeloic series This conception was supported by the improvement in association with busulphan therapy That the cells were reticulum cells was also suggested To the present author they seemed to be lymphatic (Plate II 7, 8) After final deterioration the patient died 2½ years after diagnosis The lesions found at necropsy were confined mainly to a small area of atypical tissue in and around one of the kidneys and along the ureter Histological examination gave an impression of malignant lymphogranulomatosis At check examination the material available was not considered sufficient to warrant a diagnosis Malignant lymphogranulomatosis could, however, be excluded

Case 10 is an example of a slow development of lymphosarcoma The achylia demonstrated in 1943 can hardly be taken as evidence of the disease having started already then The finding of megaloblastic anaemia, vitamin B_{12} deficiency and histamine refractory achylia in 1953 and an obscure roentgenological picture of the stomach in 1954, when an M component was demonstrated, suggests that the sarcomatous change in the stomach found at necropsy in 1960 was extensive already in the beginning of the 1950s

In case 15 the patient was operated upon because of neurinoma in the knee joint and a parathyroid adenoma Twelve years later the M component was found He then had back pain and haematemesis owing to reticulum cell sarcoma *inter alia* of the skeleton and stomach Necropsy also showed a small encapsulated renal cancer

COMMENTS

As mentioned above, nothing definite can be said about the frequency of M components in an unselected series of lymphosarcoma and reticulum cell sarcoma According to the Swedish Cancer Registry (1965), the ratio between discovered cases of chronic lymphatic leukaemia on one hand and lymphosarcoma, reticulum cell sarcoma and not specified lymphoma on the other is about 4 5 The 6 cases with M components thus represented a part of a material which was probably only slightly larger than the leukaemia group It seems therefore probable that cases with M components are more common than expected also among patients with these types of sarcomatosis

Compared with the group lymphatic

leukaemia, the course of the disease was shorter and the possibilities of observing an increase of the concentration of the M components were thus more limited. In most of the cases the concentration of the components was also fairly low. In case 10 the concentration of the M component seemed to increase slowly but continuously and favours the assumption that the M component was an early symptom of a lymphatic neoplasia developing in the course of some 10 years.

Judging from the clinical and cytological picture in cases 9 and 10 and the clinical picture and low concentration of the M component in cases 11 and 13 the diagnosis of macroglobulinaemia Waldenström did not seem to be justified in the cases with a γM component

increased and there was an increased number of mast cells.—In case 15 there were 3 % plasma cells and 1 % plasmacellular reticulum cells.

The concentration of the M component was 1.0 g/100 ml or more in only 2 cases (Table X). The component was seen at the first electrophoretic examination except in case 9, in which this examination was not technically successful (1953). Increase in the concentration was noted in case 10 (0.3 to 0.8 g/100 ml in 4 years) and in case 12 (0.4 to 1.0 g/100 ml in 6 months).

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As mentioned above nothing definite can be said about the frequency of M components in an unselected series of lymphosarcoma and reticulum cell sarcoma. According to the Swedish Cancer Registry (1965), the ratio between discovered cases of chronic lymphatic leukaemia on one hand and lymphosarcoma, reticulum cell sarcoma and not specified lymphoma on the other is about 4:5. The 6 cases with M components thus represented a part of a material which was probably only slightly larger than the leukaemia group. It seems therefore probable that cases with M components are more common than expected also among patients with these types of sarcomatosis.

Compared with the group lymphatic

Table XI *Fourteen cases in which the M component disappeared*

Case	Sex Age	Gamma fraction[1] a (g/100 ml)	b	c (g/100 ml)	M-component[2]		Time[3] I (yr mth)	II	Diagnosis
27	M 61	1 3	1 8	1 8	1 0	?	3—9	0—0	Sinusitis chron bronchitis
28	M 65	1 6	1 5	1 0	1 0	γG	0—2	1—8	Viral infect and/or allergic react
42	F 54	1 2	1 5	1 0	0 6	γG	0—1	5—2	Glandular fever
45a	M 58	3 5	4 1	2 7	0 6	?	0—8	3—3	Hyperglobulinaemic lymphoreticulosis
47	F 79	1 4	1 0	1 2	0 4	γG	0—11	1—4	Fever of unknown origin
55	F 62	1 5	1 8	2 2	0 4	γG	2—8	0—10	Encephalitis rheum arthritis
62	F 50	0 5	0 9	0 9	0 2	γG	2—1	0—0	Peritonitis
80†	M 55	0 9	0 8	1 3	0 9	γG	1—2	0—3	Polyarteritis nodosa
83†	M 63	1 1	1 3	1 6	0 9	γG	3—1	0—1	Liver cirrhosis
96†	M 48	1 1	1 3	1 3	0 5	γG	0—1	0—4	Tuberculosis
108†	F 78	0 6	0 8	0 7	0 2	γG	0—8	0—3	Melanosarcoma
IX	M 50	0 6	1 2	1 0	0 8	γM	0—1	2—11	Rheum arthritis
X	F 71	0 5	0 7	0 7	0 2	γM	2—5	0—0	Cholecystitis
XIII	M 22	1 4	1 5	1 5	0 2	γM	0—1	0—0	Malaria

[1] At highest M component concentration noted (a) at 1st electrophoresis showing no such component (b) and at last electrophoresis (c)
[2] Highest M-component concentration noted and immunological type
[3] Interval between finding of M component and 1st electrophoresis showing no such component (I) interval between last mentioned electrophoresis and last after-examination or death (II)

with agar gel electrophoresis but no M component was found

The interval between the discovery of the M component and its disappearance and between the latter and follow up varied from case to case In case 27 the interval between the last electrophoresis at which the M component was demonstrated and the following electrophoretic examination was one year and 6 months The corresponding interval was one month in cases 28 47 and 83 Otherwise the interval was that given in Table XI as the interval between the discovery and first electrophoresis showing no component

Therapy in the form of hydrocortisone prednisone or ACTH was given in cases 28 45a 80 83 and 96 In case 28 where the component disappeared after 2 months no component was demonstrable 2 years after cessation of therapy either (hydrocortisone 150—25 mg/day for 3 weeks) —In case 45a the component appeared after 2 weeks treatment with prednisone (30 mg/day) which was continued at re examination 8 months later the component was no longer demonstrable —In case 80 the concentration of the component decreased from 0 9 to 0 5 g/100 ml in 3 months Afterwards prednisone (10 mg/day) was started At re examination 10 months later the concentration was 0 2 g/100 ml Prednisone was then replaced by ACTH and after one month no M component was demonstrable —In case 83 the concentration first increased from 0 5 to 0 9 g/100 ml in one year Prednisone therapy (10—15 mg/day) was then started and the concentration decreased successively to nil within 2 years and 3 month —Case 96 had been treated with prednisone (30 mg/day) for one month before electrophoresis had been performed and an M component was discovered Therapy was continued and in one month the component had disappeared

In case 108 the M component disappeared during treatment with cyclophosphamide

In case XIII the malaria was cured with chloroquine and during this treat

CHAPTER VI

CASES IN WHICH M-COMPONENTS DISAPPEARED

The concentration of an M component in the serum, as mentioned in the previous chapters, either remains constant, as in the majority of cases in *group I*, or increases as in the myelomatosis group. Disappearance of the M component is rare, and was not reported by any of the authors referred to in Chapter I.

Anderson & Ferriman (1960) reported a case of macroglobulinaemia Waldenström where both the clinical symptoms and the M component disappeared in association with a cure with nitrogen mustard. After 18 months the patient returned to work, and at re-examination 4 years later he was in a good general condition and the serum electrophoretic pattern was normal. During the interval he had not received any treatment.

In Brackenridge & Csillag's (1962) series one man had peptic ulcer, cryoglobulinaemia and an M component (0.8 g/100 ml), which could not be seen at control 6 months later.

Clubb et al. (1964) reported a patient with hyperparathyroidism and a discrete component in the serum. At examination one month after parathyroidectomy the component had disappeared.

Nutter & Kramer (1965) reported a case with enlargement of the lymph nodes and spleen, a hyperviscosity syndrome, a cryoglobulin and probably an M component (9.8 S) in the serum. The patient improved spontaneously and examination 11 months later revealed no abnormalities.

Osserman & Takatsuki (1965) described a patient who in association with sulpha therapy developed a serious clinical picture with *inter alia* granulocytopenia, 15 % plasma cells in the bone marrow, an M component (2.6 g/100 ml) in the serum and a light-chain component in the urine. After withdrawal of sulpha the picture successively became normal. After 3 months there was no longer any proteinuria and after half a year no M component was demonstrable in the serum.

Schobel & Wewalka (1965) reported a case with diffuse symptoms and a γG-component in the serum. At re-examination 2 years later the M component was no longer demonstrable and the patient appeared to be in good health.

RESULTS

The M component disappeared in 14 subjects of the Malmö series (Table XI).

Cases 80 and 96 had bone marrow plasmocytosis (10 %, 21 %) which in case 96 was accompanied by pancytopenia, which made it difficult to judge the relative value.

The concentration of the gamma fraction was subnormal (< 0.8 g/100 ml) in 4 cases and above the upper limit of the normal range (1.3 g/100 ml) in 5. When the M component had disappeared the concentration of the gamma fraction had increased slightly in some cases. In cases 27, 45a and 55 the concentration of this fraction was high long after the component had disappeared. In cases 28 and 42 the gamma fraction concentration was normal at last examination, case XIII was not examined after the convalescence.

The concentration of the M component was in no case more than 1.0 g/100 ml. At after-examination in 1965 sera from 7 of these subjects were analysed also

state of health was good during 3 years' follow up except for sequelae after a hemiplegia 20 years previously

COMMENTS

Judging from paper electrophoresis the M components disappeared. Some sera were examined also by agar gel electrophoresis which gives a better resolution (Wieme 1959 p 75) but which did not show any discrete component either. The concentration of the gamma fraction was sometimes higher when the M component had disappeared but not so much higher as to give reason to suppose that the component was concealed.

In 5 cases the concentration of the gamma fraction was somewhat increased, which gives some support to the assumption that the M component in these cases was part of a general immunological reaction.

It is difficult to say how long it took the M component to disappear because the intervals between successive electrophoretic examinations were usually long. In some cases however the component was found to disappear in a short time (1–2 months).

It is known that cytostatics can reduce the concentration of the M component in macroglobulinaemia Waldenström (Bouroncle et al 1964) and myelomatosis (Alwall 1952, Korst et al 1964, Waldenström 1964 b) and that corticosteroids sometimes have the same effect in myelomatosis (Effersoe et al 1952, Videbæk & Harboe 1958). The course in those cases treated with corticosteroids or ACTH lend no certain support to the assumption that the therapy had caused the M components to disappear. In case 80 and to a certain extent 45a the course seemed to argue against such a correlation and in case 28 the disappearance of the M component seemed to be a sign of general recovery rather than a result of therapy.

The patients' diseases were usually of a type in which the activity of the antibody synthesising organ is increased (infectious diseases, polyarteritis nodosa, liver cirrhosis, rheumatoid arthritis). In cases resembling those discussed here, the concentration of the M components remains fairly constant for years (Chapter III IV). Since in many cases (27, 28, 42, 47, 55, 62, X and XIII) the M component was found to have disappeared after recovery, it was tempting to assume that the production of M component protein in some of these cases was part of an increased activity of the antibody producing organ, an activity which ceased when the agent causing the disease was neutralised.

The diagnosis in cases 28, 45a, 47 and 55 was difficult to make. The symptoms in case 28 were conceived by physician in charge as being due to a virus and/or an allergic reaction, and in case 55 encephalitis was suspected. In case 45a the picture was in many respects like that which Jahnke & Scholtan (1960 p 86) called hyperglobulinaemic lymphoreticulosis.

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The clinical course in cases 80 (polyarteritis nodosa), 83 (liver cirrhosis), 96 (disseminated tuberculosis) and 108 (melanosarcoma) was down hill and all 4 patients died from the disease for which they were being treated when the M component concentration was highest. In cases 45a and IV the clinical picture at after-examination after 4 and 3 years respectively was much better. Otherwise the patients' state of health was good at the after-examination or the patients had diseases other than those for which they had been treated when the M component was found.

Nine of the patients had various infections at the time of discovery of the M component. The etiologically obscure clinical picture in case 28 was thought to be the result of a virus infection and/or an allergic reaction. The picture was dominated by confusion, fever and a rash.—In case 47 fever of unknown origin developed after an arthroplastic operation because of necrosis of the femoral head following fracture. The R.B.C. was 3.0 mill. and the W.B.C. 2,400/mm³ with normal distribution. Electrophoresis showed besides an M component, hypoalbuminaemia (3.1 g/100 ml) and increased concentration of the alpha fractions. The condition gradually improved and at the last after-examination only anaemia and senile confusion persisted.—In case 55 electrophoresis had been performed one year before the finding of the M component because of E.S.R. 71 mm/1 hr and rheumatoid arthritis. The woman fell ill with headache, vomiting, fever (40°C) and impaired vision. The C.S.F. contained 82 mg protein per 100 ml and 27 mononuclear and 2 polynuclear cells per mm³. The patient also had left-sided homonymous hemianopia and the E.E.G. showed theta delta activity occipito-temporally to the right. The symptoms abated and they were believed to have been due to encephalitis.—Patient No. 62 had peritonitis secondary to infected endometriosis.—In case 96 necropsy revealed fresh tuberculous foci in the lungs, lymph nodes, spleen, liver and colon.

Of the other cases, the following deserve commentation. In case 45a a long and complicated history (given in detail by Waldenstrom 1962, case F.K.) was dominated by diarrhoea, enlargement of lymph nodes and of the spleen and polyclonal hypergammaglobulinaemia. There was lymphocytic infiltration of lymph nodes, spleen and bone marrow. Splenectomy produced no change in the picture. Soon after institution of prednisone an M component appeared. The patient improved and therapy was continued. Eight months later the component had disappeared and had not been seen during the following 3 years.

In case 83 the discrete component was found when the patient sought advice for an ulcer on the tongue. The clinical picture was later dominated by symptoms and signs of liver cirrhosis, principally ascites and deterioration. For 2½ years he was treated with prednisone, but the M component did not disappear until one month before death.

Patient IV had had a spell of arthritis 4 years before he fell ill with fever and arthritis, and 2 M components (γM, 0.4 + 0.4 g/100 ml) were found. After one month the clinical picture was unchanged, except for the fever which had begun to abate and the M components which had disappeared subsequently he made a partial recovery.

In addition to the above 14 cases mention might be made of case 19, where the concentration of the M component decreased from 1.2 to 0.2 g/100 ml in 3 years. The component which was found at a frequency study also appeared in the urine (p. 40). Her

from the prostatic cancer in case 3 A More than 3% plasma cells but of normal appearance were seen in the bone marrow smears in 2 cases In case 4 A (γM) 20% lymphocytes were seen including some atypical ones (Chapter IV)

Severe hypoalbuminaemia (3 0 g/100 ml) was seen in the man with prostatic cancer The concentration of the gamma fraction was decreased in 2 cases The concentration of the M components varied between 0 3 and 1 2 g/100 ml and their immunological type was γG in 5, γA in 2 γM in one Case 2 A showed an atypia discussed in p 40 A component with the same rate of migration was demonstrated in the urine but there was no albuminuria A light chain component in the urine was seen only in case 3 A with prostatic cancer and skeletal metastases

Eight subjects died 4 months to 2 years and 7 months after the first examination One patient (6 A) was transferred to a mental hospital where he soon died necropsy was not done and the cause of death according to the death certificate was senile dementia with pneumonia The most common finding at necropsy of the other patients was cancer Case 4 A showed no signs of recurrence of the previously extirpated gastric cancer In case 8 A it could not be decided whether the canceromatous lesions in the liver were primary or secondary In case 1 A sarcoidosis like epithelioid cell foci were seen in the myocardium lungs and lymph nodes as well as incipient cirrhosis In 2 cases the picture was dominated by *arteriosclerosis* and in one (9 A) also by pronounced cardiac amyloidosis In none of the cases did the appearance of the bone marrow suggest myelomatosis

One patient (2 A) was living and was after examined 3 years after the M component had been found She was still in a good condition and the cytological picture was unchanged, but the concentration of the discrete component in the serum and in the urine was lower (serum component 0 2 g/100 ml) X ray showed osteoporosis of the spine and compression of some vertebrae

Group B —Serum M components were found in 7 women and 2 men In 3 cases (3 B 4 B and 7 B) the presence of an M component had been known previously

At the time of the first examination one subject (3 B) had been operated upon for breast cancer 1½ years previously She was tired had anorexia, constipation alternating with diarrhoea intestinal bleeding and was bedridden One (4 B) had been treated for uterine cancer Otherwise symptoms and signs of arteriosclerosis were predominant One woman (7 B) had also for 8 years shown an obscure diffuse picture with fatigue cystopyelitis arthralgia myalgia and kerato conjunctivitis sicca

Roentgen examination of the skull, spine and pelvis was done in all cases except Nos 1 B 5 B and 9 B No lesions suggesting myelomatosis were seen In case 4 B with increased alkaline phosphatase changes suggesting Paget's disease were seen

Anaemia was common The bone marrow smears contained more than 3% plasma cells in one (3 B) of 6 cases examined In smears from cases 2 B and 6 B (γM) 10% lymphocytes respectively 22% lymphocytes and an increased number of mast cells were seen

Hypoalbuminaemia was seen in 5 case A clearly decreased concentration of the gamma fraction was noted in case 3 B The concentration of the M components varied between 0 2 and 3 1 g/100 ml and their immunological type was γG in 5 cases γA in one and γM in 3 In case 4 B the type of the component could not be determined *In 3 cases (2 B, 5 B 6 B)* the patients had not proteinuria in 5 urine electro

CHAPTER VII

FREQUENCY OF M-COMPONENTS IN SERUM OF SUBJECTS ABOVE 70 YEARS AND AFTER-EXAMINATION OF THOSE WITH AN M-COMPONENT

In 1961 when the present investigation was started we had the impression that M-components occurred above all in the high age classes To get a fair yield from a reasonably large primary series the lower age limit was set at 70 years

Part of the material (group A, see below) has been described previously (Hallen 1963) Group A was supplemented by patients admitted to an infirmary (group B) This was done partly to increase the series of subjects with M components and partly to get an opinion of the frequency of this abnormality and its possible relation to certain clinical conditions in a group which from a point of care is becoming more and more important

Both groups have now been followed up so long that a new report of group A and a description of group B appear legitimate

MATERIAL

Group A —The group consisted of sera from all persons above 70 years of age living at the 2 old age homes at Malmo with the exception of those who were unwilling to co operate or who were bedridden because of some acute disease People are admitted to these homes on social grounds and not for medical reasons Illness is if anything a contra indication for admission since these homes accept only persons able to take care of themselves In order to secure a larger material sera were also collected from 40 patients likewise at least 70 years of age at 2 female departments for senile dementia of the mental hospital in Malmo The material consisted of 294 persons—135 men and 159 women The mean age was 81 years in both sexes

Group B —The group consisted of sera from 277 *consecutive patients above* 70 years and cared for at an infirmary The 39 patients who had been examined with serum electrophoresis less than 2 years before the present investigation were not re examined The panorama of the diseases in patients above 70 years at the infirmary was dominated by cerebrovascular and cardiovascular diseases senile dementia malignant neoplasms and fractures requiring long after care Of these 277 patients 103 were males and 174 were females The mean age was 81 years males 82 females 81 years

RESULTS

Group A —Serum M components were found in 6 men and 3 women (see *Table XII* which gives data on subjects in group A and B)

At the time of the first examination three (3 A, 4 A, 5 A) had or had had cancer One (3 A) died from prostatic cancer 8 months after discovery of the M component One man (4 A) had been operated upon for gastric cancer 5 years previously, but felt well In the third (5 A) a basal cell cancer had been cured with roentgen radiation 2 years previously

One subject (1 A) had senile dementia, one (2 A) had sequelae after a cerebral lesion, which was *conceived* as a surgical complication, one (6 A) had symptoms suggesting tabes dorsalis, and one (7 A) had emphysema and clubbed fingers None had enlargement of the liver, lymph nodes or spleen

Skeletal x ray was done in all cases except 3 A In subjects 2 A, 5 A, 8 A only the skull was examined No osteolytic lesions were seen

A low R B C was noted especially in cases 2 A, 3 A and 9 A Case 2 A has *been included in* Table I, p 19 Case 3 A had prostatic cancer In case 9 A only one determination was made, Hb was 12 8 g/100 ml and unchanged at last examination 2½ years later

Histological examination of the bone marrow showed widespread metastases

study data given are findings at detection of M-component

Diagnosis or state of health at detection	Diagno is or state of health at last after examination or necrop y findings
Senile dementia	Arterioscler sarcoidosis
Hemiplegia	Hemiplegia
Cardio cler sciatica'	Prostatic cancer
Healthy	Hepatic and prostatic cancer
Senility	Arterio cler chron cholecystitis
Senility tabes dor alis	No necropsy
Emphy ema	Pulmonary cancer
Fairly healthy	Liver cancer
Healthy	Encephalomal cardiac amyloid
Cy topyelitis pleur empyema gangraena	Cystopyelit empyema cardiac amyloid
Senile dementia	Senile dementia
See ca e report	Fairly healthy
Arterio cler uterine cancer	No necropsy
Arterioscler deterior	Encephalomal
Arterioscler deterior	Encephalomal
Arterioscler etc	Arterioscler liver cirrh
Senile dementia	Senile dementia
Senile dementia	Encephalomal liver cirrh et cancer

(Axel on et al 1966) revealed 19 cases (2 5 %) with M components among 747 persons above 70 years From these re ults and from tho e presented here it is clear that serum M components must be expected in about 3% of persons above 70 years

In none of the 18 cases accounted for here had clinical cytological or roent genological examination revealed signs of myelomatosis at the time of discovery of the M components The existence of latent myelomato is could however not be excluded but necropsy of 12 subjects on the average one and a half year after the di covery of the M components revealed no signs of myelomatosis Nor did clinical cytological and roentgeno logical examination of 4 cases 2 to 3 years later Case 3 B with a relatively high concentration of the M component and increa ed number of plasma cells in the bone marrow wa however difficult to judge in this respect (p 42)

None of the 4 subjects with γM component showed the picture of macro globulinaemia Waldenström and the concentration of the M components in these cases were not high either (≤ 1 3 g/100 ml) Two of the 3 patients exam ined however, had a somewhat increased number of lymphocytes and of mast cells (one case) in the bone marrow

Especially 3 patients (1 B 7 B 9 B) had had repeated infections of the urinary tract and/or lungs But none of them had defective antibody synthesis as far as can be judged from the con centration of the gamma fraction The clinical picture and the final diagnosis varied so widely apart from the expected high frequency of geriatric disea es that no *correlation* could be demonstrated between the clinical cour e or the diagnosis and the occurrence of an M component

Table XII Subjects with M components found in a frequency

Case[1]		Sex Age	R B C (mill)	Bone marrow plasma cells (%)	Albumin (g/100 ml)	Gamma fraction (g/100 ml)	M component (g/100 ml)	Observation time (yr mth)
1 A†	75	F 83	4 2	7	4 4	1 1	0 9 γG	2—0
2 A	19	F 70	3 7	4	4 1	1 3	1 2 ?	3—0
3 A†	105	M 82	3 4		3 0	0 2	0 3 γA	0—8
4 A†	XXIII	M 79	5 2	1	4 8	0 8	0 7 γM	2—1
5 A†	78	F 89	4 0	2	4 6	0 8	1 0 γG	1—4
6 A†	O O	M 80	4 3	<1	5 2	0 6	1 1 γG	1—1
7 A†	90	M 89	4 3	1	4 9	0 9	0 7 γA	1—5
8 A†	104	M 90	4 1	1	4 5	1 0	0 3 γG	1—2
9 A†	84	M 87	3 6	1	5 0	0 8	0 8 γG	2—7
1 B†	XXI	F 85	4 2		3 4	1 0	1 3 γM	0—3
2 B	XI	F 83	4 6	2	4 2	0 7	0 2 γM	2—2
3 B	1	F 84	3 2	8	3 2	0 5	3 1 γG	2—5
4 B†	E N	F 85	3 0	1	3 0	1 4	0 4 ?	0—3
5 B†	89	M 89	4 4		3 4	0 9	0 8 γG	0—2
6 B†	XXV	M 78	3 4	1	4 8	0 8	0 6 γM	0—5
7 B†	67	F 72	3 1	2	2 0	1 3	1 5 γG	0—7
8 B	61	F 81	3 2		4 3	1 0	0 2 γA	2—3
9 B†	85	F 77	4 2		4 8	1 1	0 9 γG	0—2

[1] Second column gives case number in Tables VIII and XIII Initials are given for patients not included in these tables

* Intervals between detection at frequency study and last after examination or death

phoresis showed no light chain component, and in case 4 B proteinuria was not satisfactorily excluded

Six patients died 2 to 7 months after the first examination Necropsy was performed in 5 in whom the picture was dominated by changes due to age and infectious diseases Two women were found to have liver cirrhosis and in one of them observations made in one area suggested incipient liver cancer

The remaining patients were examined about 2½ years after discovery of the M component The picture was clinically, cytologically and biochemically largely unchanged in cases 2 B and 8 B In case 3 B it was considerably improved The patient was no longer bedridden and diarrhoea and intestinal bleeding had ceased The concentration of the M component was lower (2 5 g/100 ml) Her condition has since deteriorated (p 42)

COMMENTS

As previously mentioned (Hällen 1963), group A may be regarded as a largely random selection of elderly subjects In group B, on the other hand, the general condition of many of the subjects was poor Of this group 39 in whom serum electrophoresis had been performed less than 2 years previously were not re examined Whether this omission is justified is open to discussion It does not, however, seem likely that M components would have developed in so many of these 39 cases as to influence the frequency of M components found

Fine et al (1965) discovered 16 cases of M components among 500 subjects above 68 years A population study

events but does not exclude the possibility of the cases representing myelomatosis in an early but stationary stage e g as in case 72m Such cases are, however so rare (Chapter III) that group I can surely include only a very small number of them

The relation between the "essential" cases with M component of type γM and macroglobulinaemia Waldenström is more difficult to decide The cases in the present series were few and followed for only a relatively short time in view of the usually slow progression of macroglobulinaemia Waldenström Nothing argues, however against some of the cases with γM component like those with γG or γA component being stationary and never developing the clinical picture of macroglobulinaemia Waldenström

Summarising there is strong evidence of the existence of benign essential monoclonal gammapathy i e a condition in which a non increasing activity of gammaglobulin synthesising cells manifests itself as an M component but where a clinical picture of a neoplastic disease of plasma lympho or reticulo cellular origin does not exist and in which the M component itself does not give rise to conditions such as haemolytic anaemia or Raynaud syndrome

What possibilities are there of deciding whether a protein change in a given case should be regarded as "essential"?

The clinical picture and course of myelomatosis (mainly γG and γA component) differs so strikingly from macroglobulinaemia Waldenström (γM) that each condition is discussed separately

Since progression is characteristic of *myelomatosis* it was natural to expect that the concentration of the M components in this group should be higher than that commonly seen in group I This was also found to be so in the present material The 2 groups, however overlapped in the region below 3 0 g/100 ml

The question then arises to what extent other symptoms or signs can facilitate the differential diagnosis in cases with an M component but without osteolytic lesions or severe (>20 %) bone marrow plasmocytosis

Loss of bodyweight was common in the myelomatosis group but not among those cases where the diagnosis was initially uncertain

Anaemia is common in myelomatosis and should thus be a sign of diagnostic value In half of the patients with serum M component of low or moderate concentration but no light chain component this sign was however missing Like weight loss anaemia is an unspecific sign and as most of the subjects in group I were discovered when they sought medical advice these 2 signs were not infrequently found in that group too

Bone marrow plasmocytosis above 10 % was rare in group I and was seen *inter alia* in a few cases where myelomatosis was suspected sometimes strongly so Values below this level were however seen in several cases in the myelomatosis group Even though many of these patients showed osteolytic lesions and though the smallness of the number of plasma cells could sometimes be explained by less successful punctures some of the cases illustrated the difficulties in making a diagnosis Abundant atypical cells were most common in the myelomatosis group In some cases however the cells were morphologically normal and in group I there were some cases with clearly atypical cells To base the differential diagnosis solely on the presence or absence of "myeloma cells" is thus not possible

Also concerning the concentration of serum albumin and the gamma fraction it was difficult to draw a line of distinc

CHAPTER VIII
CONCLUDING REMARKS

An M component in the serum sometimes has a clinical and cytological *counterpart, above all* myelomatosis or macroglobulinaemia Waldenstrom. Many authors feel that there is a relation also between the occurrence of M components and chronic lymphatic leukaemia, lymphosarcoma and reticulum cell sarcoma. If cases with an M-component cannot be assigned to any of these 5 groups of diseases (sometimes the 3 last mentioned ones have not been included) the condition has been classified under different names such as benign, essential monoclonal hyperglobulinaemia or gammapathy, dysproteinemies a caractere atypique and kryptogenetische, symptomarme or idiopathische Paraproteinämie (Chapter I).

Are "essential" cases more common or less common than myelomatosis or macroglobulinaemia Waldenstrom?

The result of the frequency study mentioned in chapter VII shows that in a group with M components the "essential" cases are by far most common. A similar result was obtained in a population study (Axelsson et al. 1966), the diagnosis of myelomatosis was more or less strongly suspected in 3 of 64 subjects with serum M components and the clinical picture of macroglobulinaemia Waldenstrom was not seen in any of the 7 subjects with a γM component. To what extent the remaining 61 cases represented early forms of myelomatosis or macroglobulinaemia Waldenstrom is a question that must abide an after-examination.

Summarising, "essential" cases are much more common than myelomatosis and macroglobulinaemia Waldenstrom. This difference is, however, surely to some extent due to the shortness of the survival time in myelomatosis. Since 2—3 % of the subjects in the age groups in which they are especially liable to come into contact with hospital have serum M components, it is necessary to be able to evaluate this finding.

Should "essential" cases be conceived as cases of myelomatosis or macroglobulinaemia Waldenstrom in an early stage?

The concentration of the M component has been shown to vary with the size of the tumour mass (the number of plasma cells) not only in mouse plasmocytoma (Nathans et al. 1958), but also to some extent in human myelomatosis (Märki & Wuhrmann 1965). An increase of the concentration of an M component should thus reflect an increase of the cell mass. Repeated follow up with electrophoresis, then, provides a possibility of deciding whether and at what rate the condition progresses (Waldenstrom 1964 a).

The development of myelomatosis can occasionally, at least for a time, be very slow, and several years may elapse before accelerated progression reveals the true nature of the condition (Chapter III). If an M component is incidentally discovered in such a case without other signs of myelomatosis, the case must be conceived as "essential". If group I consisted of such cases even a very slow progress should be reflected by an increased number of cases with increasing positive difference with increasing observation period in Fig. 9, p. 33. Fig. 9 argues against such a sequence of

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tion between the 2 groups Normal values may be regarded as arguing against, but not excluding, myelomatosis, and subnormal values, particularly of the gamma fraction, are compatible with "essential" gammapathy

The immunological type of the M-component (γG or γA), or demonstration of an anticomplementary activity of the serum was of no diagnostic significance

The finding of a light-chain component in the urine argues strongly for myelomatosis In two thirds of the myelomatosis group, however, no such finding was made in the early stage In group I it was rare

Thus, in a few cases with M components it may be difficult to decide immediately whether myelomatosis is present or not Follow up of the patients is then of importance because the clinical picture of myelomatosis usually progresses rapidly and the concentration of the M component increases In none of the cases in the myelomatosis group was a clear spontaneous decrease of the concentration of the component noted which, however, had been reported by Marki et al (1965) Such a sequence of events was seen in case 1 in group I which was a borderline case from a diagnostic point of view Slow courses were, however noted in a few cases in the myelomatosis group, the M component remaining unchanged or increasing only slightly in concentration for years

The interval between the re examinations and the length of the follow up in obscure cases will of course be dependent on the medical facilities available In view of the usually rapid progression of myelomatosis it would however appear advisable to re examine a subject with an M component 2 to 3 months after discovery and then after a further 3 to 6 months depending on the presence or absence of an increase in the concentration of the M component In case of increase the patient should be examined for osteolytic lesions or rapidly progressing osteoporosis of the spine The possibility of influencing the course of the disease by *e g* melphalan are considerable (Waldenstrom 1964 b) and in case 62m (p 14) the period with paraplegia could probably have been prevented if the disease had been discovered and treated earlier Development of skeletal lesions without simultaneous increase of the concentration of the M component is rare (case 58m) If the concentration is constant, it will be difficult to decide when follow up should be stopped In most subjects with an M component in the serum this protein will probably persist in unchanged concentration without the patient developing myelomatosis But cases have been described in the literature and were seen in the present myelomatosis series where the concentration of an M component was low (0 3 g/100 ml in case 72m) and constant for several years and then suddenly increased in association with the appearance of other signs of myelomatosis Such cases are, however, very rare

To what extent should the patient be informed of the finding of an M component? Even if there is no reason to discuss the possibility of myelomatosis it seems advisable to tell the patient that there is an increase of the E S R and ask him to return for control in the event of any symptoms particularly (skeletal) pain But even such information must often cause unnecessary anxiety On the other hand, if the patient is kept in ignorance, as in case 40m the consequence may be disastrous

It is often more difficult to decide whether a subject with a γM component should be regarded as having *macroglobulinaemia Waldenstrom* This is above all because this disease progresses much more slowly than myelomatosis The importance of an early diagnosis on the other hand, seems not to be of such

great practical importance as in myelomatosis It is true that cytostatic therapy can be offered but such complications as those following osteolytic lesions in myelomatosis need not be feared As in chronic lymphatic leukaemia a disease related in several respects to macroglobulinaemia Waldenström (Waldenström 1960) the clinical picture decides whether treatment should be given or not

Can M components be related to diseases other than myelomatosis or macroglobulinaemia Waldenstrom?

In chapter V it was shown that the frequency of M components in an unselected series of chronic lymphatic leukaemia was larger than expected In some of these cases the concentration of the M component was found to increase and in some of them the component appeared during the course of the disease Such observations naturally provide further support for the assumption *of a relation* between the disease and protein changes Also in lymphosarcoma and reticulum cell sarcoma M components are possibly more common than expected

The relation if any between other diseases and M components is difficult to evaluate (Chapter III IV) In those cases however where an M component develops in association with the onset of a disease or disappears with the recovery the relation between the disease and the protein change appears probable Only a few such cases have been described In the cases where the M component disappeared (Chapter VI) there were above all more or less well defined infectious diseases polyarteritis nodosa liver cirrhosis and rheumatoid arthritis In some cases the interval between the electrophoretic studies was so long that it could not be decided whether the M component had disappeared in association with recovery of the patient In a few cases, however, the M component disappeared spontaneously during recovery This and the types of diseases represented argue for the component sometimes being part of an antibody response to an infectious agent for example

The rest of the material leaves the impression that the activity of an M component producing clone either reaches a certain level and then remains constant or increases more or less rapidly and gives rise to a well defined clinical picture (myelomatosis, macroglobulinaemia Waldenström)

It is not known what triggers off the synthesis of an M component It is, however tempting to assume some agent that can stimulate the antibody synthesising organ *e g* agents which produce infectious diseases or allergic reactions possibly also neoplasms which can contain proteins with antigenic determinants not detectable in normal tissue (see Tee et al 1964) Of interest in this connection is that reticulum cell tumours have been shown to develop *in mice* after antigenic stimulation (Metcalf 1961 Schwartz & Beldotti 1965) and Talal & Bunim s (1964) report on the development of malignant lymphomas in a few cases and a γM component in one, all interpreted as suffering from Sjögren s syndrome Further in an inbred strain of mice with polyclonal hypergammaglobulinaemia haemolytic anaemia and glomerulonephritis Mellors *(1966) noted* the development of lymphomas and at the same time in some animals a transition *to a monoclonal* hypergammaglobulinaemia In view of the history and state of *health of the* 64 subjects with M components found in a population study (Axelsson et al 1966) such a stimulating factor need not however, *cause* pronounced or characteristic symptoms Only a few subjects possibly with *predisposing* heredity (Chapter III) would especially at an advanced age

respond in this special way to a stimulus Particularly among the aged, antibodies against the gastric mucosa (Irvine 1965) or rheumatoid factor like globulins (Aron et al 1961, Heimer et al 1963) have been found without corresponding clinical symptoms This, together with the high mean age of patients with myelomatosis and macroglobulinaemia Waldenstrom and the fact that the number of subjects with M components in a normal popula tion increases above 50 years of age suggest a lability of the antibody producing organ in the aged

SUMMARY

The purposes of the present investigation of a series of cases with M components in the serum were to ascertain whether certain cases ("essential") do not develop the clinical picture of myelomatosis or macroglobulinaemia Waldenström, whether such "essential" cases are common and thereby present an important diagnostic problem and if so how to solve it, whether serum M components are related to diseases other than those mentioned above and whether the component in such case is of prognostic or diagnostic significance.

Chapter III is concerned with 108 cases (group I) with components which were not of type γM and in which the subjects did not fulfil the diagnostic criteria of myelomatosis used and apparently had not leukaemia, lymphosarcoma or reticulum cell sarcoma. The observation period was on the average 3 years and all except 24 of those who had died had been observed for one year or more. Forty six subjects had died and had been studied post mortem with histological examination of at least one part of the skeleton. — An unselected series of 92 patients with myelomatosis was studied with regard to the clinical, cytological and biochemical picture they developed within one year after diagnosis or detection of the M component or before cytostatics were instituted.

The age distribution in group I was largely the same as in the myelomatosis group (mean age about 70 years). Group I consisted of 52 men and 56 women. In the myelomatosis group there were 37 men and 55 women. This sex distribution was that statistically expected assuming that men and women are equally liable to develop myelomatosis.

Anaemia (male R.B.C. < 4.2, female R.B.C. < 3.8 mill.) was noted in 17 % of 51 subjects in group I taking part in the last after-examination, while an R.B.C. at or above this level was noted in 17 % of the patients in the myelomatosis group. In some of the 17 % in the myelomatosis group also other signs were missing.

In 46 % of group I the highest values noted for bone marrow plasma cells were more than 3 % and 9 % had more than 10 % plasma cells. In the myelomatosis group 24 % of the patients had less than 10 % of such cells. Subjects with atypical plasma cells were more common in the myelomatosis group. Atypical forms were, however, also seen in smears from some subjects in group I and mostly normal cells in smears from some patients with myelomatosis.

A subnormal concentration of serum albumin was seen in 12 % of 51 largely healthy subjects in group I and particularly in those in whom the concentration of the M component exceeded 1.5 g/100 ml. The concentration was normal in 24 % of the myelomatosis group and particularly among those with a low M component concentration.

The concentration of the gamma fraction (excluding any M components with gamma mobility) was subnormal in 46 % of group I and in 26 % it was equal to or less than the average level in the myelomatosis group (0.5 g/100 ml).

The concentration of the M component was more than 1.0 g/100 ml in 32 % of group I and more than 2.0 g/100 ml in 11 %. As a rule, the concentration

respond in this special way to a stimulus Particularly among the aged, antibodies against the gastric mucosa (Irvine 1965) or rheumatoid factor like globulins (Aron et al 1961, Heimer et al 1963) have been found without corresponding clinical symptoms This, together with the high mean age of patients with myelomatosi and macroglobulinaemia Waldenström and the fact that the number of subjects with M components in a normal population increases above 50 years of age suggest a lability of the antibody producing organ in the aged

reticulum cells All had serum M components

The 8 patients with lymphatic leukaemia belonged to an un elected series of 59 patients with this disease The observed number of cases with M components in this series was higher than that statistically expected This appeared to be due above all to an increased number of cases with γM components In some cases the component appeared during the course of the disease and sometimes the concentration was found to increase In most cases the concentration of the M component was less than 1 0 g/100 ml The bone marrow smears from the patients with γM component did not give the impression of macroglobulinaemia Waldenström The cases with an M component in the serum were too few to allow any conclusion as to whether an M component in lymphatic leukaemia is of any prognostic significance

Also the remaining 7 cases had components above all of γM type and usually in a concentration below 1 0 g/100 ml A rough estimation suggested that also in lymphosarcoma and reticulum cell sarcoma M components were more common than expected

Chapter VI deals with 14 cases in which the M component disappeared and one in which the concentration diminished considerably Some cases had been treated with ACTH or corticosteroids The course did not give the impression that therapy was always the cause of the disappearance of the M component The concentration of the component was 1 0 g/100 ml or less In some cases the M component was shown to disappear in association with recovery of the patient The diseases which were mainly represented were such as are often accompanied by an increased activity of the antibody synthesizing organ (infectious disease liver cirrhosis polyarteritis nodosa, rheumatoid arthritis)

Chapter VII describes 18 subjects with M components discovered on electrophoretic examination of the serum from 294 largely healthy subjects above 70 years and 277 sera from patients above 70 years and cared for at an infirmary

The concentration of the components varied between 0 2 and 3 1 and in 13 it did not exceed 1 0 g/100 ml The immunological type was γG in 9, γA in 3 γM in 4 and uncertain in 2 The bone marrow contained at most 8 % plasma cells and in 2 cases with γM component abundant lymphocytes and an increased number of mast cells were noted

In none of the 18 cases could myelomatosis or macroglobulinaemia Waldenström be diagnosed Twelve of the patients were examined post mortem 3 months to 2 years and 7 months after the discovery of the M component In none of these cases was there any evidence in support of the above diagnoses nor was any such evidence found in the 4 cases after examined 2 to 3 years after discovery of the M component

CONCLUSIONS

M components are most common in the aged and occur in about 3% of subjects above 70 years

The finding *per se* of an M component is rarely tantamount to myelomatosis or macroglobulinaemia Waldenström and there is strong evidence of the existence of benign essential monoclonal gammapathy

In patients with myelomatosis and a serum M component diagnosis usually offers no difficulties A few primarily equivocal cases make it necessary to follow above all the M component concentration in subjects without evidence of myelomatosis

remained constant A correlation was found between the concentration of the M component and the relative number of plasma cells in the bone marrow In 45 % of the myelomatosis group the concentration of the component did not exceed 3 0 g/100 ml In those cases that were followed up and given only expectant treatment the concentration almost always increased rapidly

A light-chain component was found in the urine in 4 of the 90 cases studied in group I and in 1/3 of 71 cases studied in the myelomatosis group

M components of type γA represented 20 % of both group I and the myelomatosis group

An anticomplementary activity was found in 11 % of 93 sera of group I and in 15 % of 86 sera of the myelomatosis group

Some of the patients in group I had severe, progressive symptoms, but only in a few could these symptoms be ascribed to diseases believed to be related to disturbed plasma cell activity In some cases the concentration of the M component increased, in a few of these cases myelomatosis was suspected, in the others the concentration became constant after varying periods In the myelomatosis group the course was, with few exceptions, dominated by alarming symptoms such as pain and loss of bodyweight and in 50 % the survival time was one year and one month

Group I included a number of patients with co existing diseases, but it was not possible to say whether these were in any way related to the occurrence of the M component

Chapter IV describes 27 patients with γM components The mean age was 71 years and the number of males was 13

Anaemia was common but could often be explained by conditions other than macroglobulinaemia High titer cold agglutinins was noted in 2 cases

In 11 of the 20 cases studied bone marrow smears showed a more or less marked increase of the number of lymphocytes In 7 of the cases these cells strikingly often showed features of small lymphoid reticulum cells An increased number of mast cells were seen in 10 cases, while plasmocytosis was noted in only a few and then on histological examination

The concentration of the M component was more than 1 0 in 10 cases and 0 5 g/100 ml or less in 10 The concentration was usually constant

Macroglobulinaemia Waldenström was said to exist in 4 cases where a more or less progressive course could not be explained by any co existing diseases M components in a concentration above 1 0 g/100 ml and bone marrow smears characteristic of the above mentioned condition were seen also in patients with malignant tumours Two subjects with M component in low concentration (< 0 5 g/100 ml) showed cytologic features compatible with the diagnosis of macroglobulinaemia Waldenström An M component of low and constant concentration was noted in many subjects that were healthy or had diseases presumably not associated with an abnormal immunoglobulin synthesis The observation time was often long and in 5 cases there was reason to suspect that the M component had existed for more than 10 years

Malignant tumours were found in 10 cases The frequency of these tumours and the time relationship between them and the protein finding allowed no conclusions concerning a possible relation between the disease and the serum finding

Chapter V describes 8 cases of chronic lymphatic leukaemia, one case conceived as blast cell leukaemia and 6 with sarcoma built up of lymphatic or

reticulum cells. All had serum M components.

The 8 patients with lymphatic leukaemia belonged to an unselected series of 59 patients with this disease. The observed number of cases with M components in this series was higher than that statistically expected. This appeared to be due above all to an increased number of cases with γM components. In some cases the component appeared during the course of the disease and sometimes the concentration was found to increase. In most cases the concentration of the M component was less than 1.0 g/100 ml. The bone marrow smears from the patients with γM component did not give the impression of macroglobulinaemia Waldenström. The cases with an M component in the serum were too few to allow any conclusion as to whether an M component in lymphatic leukaemia is of any prognostic significance.

Also the remaining 7 cases had components above all of γM type and usually in a concentration below 1.0 g/100 ml. A rough estimation suggested that also in lymphosarcoma and reticulum cell sarcoma M components were more common than expected.

Chapter VI deals with 14 cases in which the M component disappeared and one in which the concentration diminished considerably. Some cases had been treated with ACTH or corticosteroids. The course did not give the impression that therapy was always the cause of the disappearance of the M component. The concentration of the component was 1.0 g/100 ml or less. In some cases the M component was shown to disappear in association with recovery of the patient. The diseases which were mainly represented were such as are often accompanied by an increased activity of the antibody synthesising organ (infectious disease, liver cirrhosis, polyarteritis nodosa, rheumatoid arthritis).

Chapter VII describes 18 subjects with M components discovered on electrophoretic examination of the serum from 294 largely healthy subjects above 70 years and 277 sera from patients above 70 years and cared for at an infirmary.

The concentration of the components varied between 0.2 and 3.1 and in 13 it did not exceed 1.0 g/100 ml. The immunological type was γG in 9, γA in 3, γM in 4 and uncertain in 2. The bone marrow contained at most 8 % plasma cells and in 2 cases with γM component abundant lymphocytes and an increased number of mast cells were noted.

In none of the 18 cases could myelomatosis or macroglobulinaemia Waldenström be diagnosed. Twelve of the patients were examined post mortem 3 months to 2 years and 7 months after the discovery of the M component. In none of these cases was there any evidence in support of the above diagnoses nor was any such evidence found in the 4 cases after examined 2 to 3 years after discovery of the M component.

CONCLUSIONS

M components are most common in the aged and occur in about 3 % of subjects above 70 years.

The finding *per se* of an M component is rarely tantamount to myelomatosis or macroglobulinaemia Waldenström and there is strong evidence of the existence of "benign essential monoclonal gammapathy".

In patients with myelomatosis and a serum M component diagnosis usually offers no difficulties. A few primarily equivocal cases make it necessary to follow above all the M-component concentration in subjects without evidence of myelomatosis.

remained constant. A correlation was found between the concentration of the M component and the relative number of plasma cells in the bone marrow. In 45 % of the myelomatosis group the concentration of the component did not exceed 3.0 g/100 ml. In those cases that were followed up and given only expectant treatment the concentration almost always increased rapidly.

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APPENDIX

The borderline between macroglobulinaemia Waldenstrom and benign essential monoclonal (γM) gammapathy is diffuse.

The frequency of patients with serum M components in chronic lymphatic leukaemia is higher than that expected.

The investigation allowed no definite conclusions as to whether M components are related to diseases other than myelomatosis, macroglobulinaemia Waldenstrom and chronic lymphatic leukaemia. Sometimes an M component in low concentration seems to be part of an antibody response and can occasionally disappear.

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Case 6 Female born in 1913 In 1942 she underwent cholecystectomy In 1943 and 1945 she was examined because of epigastric pain and vomiting R B C 4 5 mill E S R 8 mm/1 hr After the latter occasion she was subjected to laparotomy the operation revealed no cause of the pain which did not return From the beginning of the 1940s she occasionally had low back pain for which she sought medical advice and in 1960 she was referred to hospital R B C 3 7 mill in the bone marrow 15 % plasma cells mostly with compartment formation (Plate I 5 6) E S R 85 mm/1 hr an M-component was demonstrated in the serum (γA 2 1 g 100 ml) but the urine contained no protein Skeletal x ray showed a system of thin walled cysts in the right clavicle but otherwise nothing abnormal The woman was sent home in a good condition Later puncture of the clavicular cyst was performed but revealed nothing and histological examination of a surgical biopsy specimen was unrewarding In October 1961 her condition was unchanged but the concentration of the M-component had risen to 2 6 g 100 ml Treatment with melphalan was started and the concentration of the component fell (Fig 15 p 00) Since she was in a good condition the drug was withdrawn The concentration of the M-component then gradually increased In 1965 skeletal x ray was unchanged R B C 3 2 mill no proteinuria and the bone marrow contained 16 % plasma cells

Case 9 Male born in 1896 He had felt well until October 1962 when he was admitted to hospital because of bronchopneumonia which had been preceded by catarrhal symptoms for one month R B C 4 1 mill A serum M-component (γG 1 g 100 ml) was found and the concentration of the gamma fraction was 0 6 g 100 ml He recovered but later cough returned and in April 1963 he was admitted because of bilateral bronchopneumonia which disappeared during a 6 week spell in hospital In July 1963 he again had a cough and x ray showed bronchopneumonia At after-examination in 1965 he was found to be in a good general condition and had been so for the last 2 years He had increased in weight R B C 4 3 mill 2 % plasma cells in the bone marrow electrophoretic pattern unchanged no proteinuria no osteolytic lesions

Case 19 Female born in 1892 She had felt well until a uterine myoma which caused metrorrhagia was extirpated in 193 When she awakened after the anaesthesia hemiplegia was noted Recovery was poor Apart from the hemiplegia she was in a good general condition when her serum M-component was discovered in a frequency study in March 1962 (case 2 A in chapter VII) The M-component concentration was 1 2 g 100 ml and the component was seen in the alpha$_2$ beta$_1$ region A component with the same rate of migration was found in the urine which contained no albumin In 1963 the concentration of the M-component was 0 5 and in March 1965 it was estimated at 0 2 g/100 ml In 1965 as in 1962 her general condition was good there was anaemia of obscure origin (p 00) the bone marrow contained 4 % mature plasma cells and skeletal x ray showed no osteolytic lesions

Case 28 Male born in 1895 He had previously felt well. After 3 attacks of sinusitis in the spring of 1960 he fell ill in the beginning of April with fatigue headache muscle pain and cheels Physical examination revealed nothing remarkable except a few palm-sized erythematous patches on the thigh chest and on the upper arms R B C 4 1 mill W B C 12 400/mm^3 of which 87 % were neutrophilic leukocytes E S R 20 mm/1 hr He became sluggish auricular fibrillation supervened exanthema became generalised and here and there petechiae and vesicles appeared The C.S F contained 132 mg protein per 100 ml 46 polynuclear and 40 mononuclear cells/mm^3 but no bacteria In the serum the bilirubin was increased (1 5 mg/100 ml) and an M-component (γG 0 5 g/100 ml) was found The patient was given Actocortin® (hydrocortisone 150—25 mg day for 3 weeks) and ACTH 90—15 U for one week He improved and one month and a half after onset he was sent home in a fairly good general condition with R B C 3 3 mill and E S R 43 mm/1 hr One month later the M-component which had formerly had a concentration of 1 0 g/100 ml was no longer demonstrable and the patient was in good health

Case 35 Male born in 1902 Gastrectomy was done in 1952 (peptic ulcer) From 1957 on he had a marked tendency to infection He was admitted for the first time because of this in December 1957 after a few days common cold X ray showed bronchopneumonia R B C 4 1 mill lowest E.S R 18 mm 1 hr Afterward he had almost annually one or more attacks of catarrhal symptoms of the respiratory tract and on several occasion x ray showed broncho pneumonia and or sinusitis In December 1960 the Hb was 11 6 g 100 ml R B C 3 9 mill serum iron 0 µg 100 ml total iron binding capacity 455 µg 100 ml and the serum vitamin B$_{12}$ was low The bone marrow contained 1 % plasma cell a serum M component (γA 0 8 g/100 ml) was demonstrated and the concentration of the gamma fraction was 0 g 100 ml He was given vitamin B$_{12}$ and in 1964 the R B C was 4 5 mill At after-examination in 1965 he reported that the tendency to infection had been less marked the last year He was in a good general condition R B C 4 3 mill 3 % plasma cells in the bone

CASE REPORTS

GROUP I

Case 1 Female born in 1879 In 1961 she was operated upon for breast cancer Post operatively she complained of fatigue and anorexia There was urinary incontinence alternating constipation and diarrhoea occult blood in the stools and loss of weight Hb 10 7 g/100 ml R B C 3 5 mill in the bone marrow 8 % later 18 % plasma cells mainly of normal appearance but some large with loose chromatin and nucleoli The serum contained an M component (γG) which increa ed rapidly in concentration from 2 6 to 4 1 g/100 ml (Fig 15, p 00) Cystoscopy revealed an ugly inflammation which gradually regres ed The patient was in a poor general condition and was cared for at an infirmary In 1963 she began to improve her weight increased she could sit up and the concentration of the M component fell but anaemia persisted The Schilling test and serum vitamin B_1 concentration were normal but the folic acid concentration in the serum was low The concentration of the serum haptoglobin was normal Neither iron vitamin B_{12} or folic acid treatment had any demonstrable effect on the anaemia X ray showed that a few compression fractures of the vertebrae had occurred since 1962 At examination in the spring of 1965 the woman was in a better general condition—apart from the anaemia—and could walk unaided The concentration of the M component was 2 5 g/100 ml no light chain component was found in the urine and the bone marrow contained 3 % plasma cells In the autumn of 1965 the patient complained of fatigue and was anorectic she could not leave her bed she lost weight and the M component concentration increased to 3 3 g/100 ml

Case 2 Female born in 1875 (case H M in Waldenstrom 1964 a) She was admitted to hospital in 1927 because of cystopyelitis and in 1945 1947 and 1953 because of thrombophlebitis of the right leg The E S R on tho e occasions were 16 17 and 26 mm/1 hr In July 1959 she was admitted because of thrombosis of the right leg Physical examination revealed nothing el e of interest R B C 3 7 mill in the bone marrow 9 % plasma cells one tenth of which were plasmacellular reticulum cell and about one third of the cells had mainly small nucleoli E S R 72 mm/1 hr and a serum M component (γG 2 3 g/100 ml) was demonstrated but no proteinuria Skeletal x ray on various occa ions (last in 1965) showed no osteolytic lesion During a stay in hospital she developed pneumonia and haematemesis of obscure origin but she left hospital in a good condition in September Two months later palpation revealed an enlarged spleen extending to the umbilical plane In November 1960 she was re admitted for control She had then back pain she felt short of breath and more tired than previou ly The spleen was enlarged R B C 3 3 mill W B C and thrombocyte count normal 11 % plasma cells in the bone marrow and the concentration of the M component was 3 7 g/100 ml Treatment with melphalan was started and the concentration of the M component fell (Fig 15 p 00 lowest level recorded 0 5 g/100 ml) At follow up in 1965 she was in a good condition

Case 3 Female born in 1897 In March 1959 she fell ill acutely with fever headache and cough R B C 4 6 mill 3 % largely normal plasma cells in the bone marrow a erum M component (γG 1 2 g/100 ml) no osteolytic lesions She recovered rapidly During the following years she did well but the concentration of the M component gradually increa ed (Fig 15 p 00) In April 1964 the R B C was 4 2 mill In the end of 1964 and in the spring of 1965 she felt tired and depres ed she suffered from anorexia lost a few kilos and periodically had generalised diffuse pain At after examination in March 1965 after treatment with an antidepressive drug she was in a good general condition Her weight was unchanged R B C 3 8 mill in the bone marrow 16 % pla ma cells some of which were atypical (Plate II 5 and p 00) but no proteinuria no o teolytic le ions The concentration of the M component was 2 8 and in September 3 0 g/100 ml

Case 4 Female born in 1901 In 1913 she had rheumatic fever Diabetes mellitus was diagnosed in 1949 From 1949 to 1953 she often had infections of throat and for some time al o pain in the hand and foot joints She was admitted in 1953 because of adiposity loss of hair and hypertension R B C 5 0 mill E S R 20 mm/1 hr No evidence of impaired thyroid or adrenocortical function was produced In 1958 she was given insulin because of progres ive retinopathy R B C 5 0 mill E S R 30 mm/1 hr In 1959 the E S R was 39 and in July 1960 it wa 57 The E S R then persisted at 50—70 mm/1 hr and the M component (G) which wa discovered in 1960 remained unchanged (about 2 5 g/100 ml) until the la t control in August 1965 Hypoalbuminaemia (about 3 7 g/100 ml) was usually noted At after examination in 1965 she wa almost blind and had lo t 9 kg in the la t 3 years R B C was 4 2 mill As on 3 earlier occasions the bone marrow contained an increased number of plasma cell (14—19 %) 40 % of which exhibited vacuoli ed cytoplasm with eosinophilic pellets in mo t vacuoles and large nucleoli in three fourths of the cell (Plate I 2 3) No proteinuria serum creatinine 1 0 mg/100 ml no osteolytic le ions

concentration of the M component had increased Roentgen examination of the stomach showed nothing remarkable Her condition was afterwards dominated by abdominal pain, she deteriorated and anaemized The concentration of the M-component increased to 3 9 g/100 ml (Fig 15 p 00) but at most 3 %, plasma cells were noted in the bone marrow She died in August 1960 — Necropsy Large gastric cancer with metastases of the lymph nodes and infiltration of the right diaphragm plasma cell infiltration around the tumour and in the spleen lymph nodes and liver no bone marrow plasmocytosis concrements and pyelonephritic scars in right kidney

Case 64 Female born in 1887 Symptoms of gall stones without fever or jaundice started in 1950 In 1953 she was admitted because of pneumonia and in 1958 because of iridocyclitis The R B C was then 4 2 mill and the E S R 12 mm/1 hr In 1960 she was admitted for thrombosis of the lower leg R B C 4 1 mill in the bone marrow 11 % plasma cells with crystal-shaped holes in the cytoplasm (Plate I 1) and with nucleoli in about one fourth of the cells Serum creatinine 1 3 mg/100 ml the urine contained protein but not pus E S R 37 mm/1 hr in the serum an M component (γG 2 6 g 100 ml) X ray showed moderate osteoporosis As later slight glycosuria was occasionally observed but the blood sugar level was normal In 1961 she was admitted for examination to find out whether cytostatic treatment was indicated She was tired but otherwise her general condition was unchanged The blood values and the concentration of the M-component were largely unchanged The serum creatinine was 1 8 mg 100 ml and the urinary sediment was normal A light-chain component was demonstrated in the urine as well as albumin and other globulin fractions The bone marrow contained 3 % plasma cells of the same type as before Cytostatic treatment was not considered indicated and the patient was sent home In the spring of 1963 an attack of cystitis was curtailed by sulpha treatment When admitted in April 1964 he had been thirsty and anorectic she had lost weight and had nocturia She was emaciated R B C 3 0 mill bone marrow and M-component unchanged serum creatinine 4 2 mg 100 ml serum calcium 8 2 and phosphorus 3 7 mg 100 ml no osteomalacia or osteolytic lesion Analysis of the urine because of suspected Fanconi syndrome showed amino acid Fluid therapy produced an improvement but cholecystitis supervened and operation was considered indicated despite her poor condition After the operation the patient occasionally had intestinal bleeding and 2 months later she died i e in July 1964 Necropsy Deep ulceration particularly in the duodenum and together with unspecific inflammation also in the tongue oesophagus and rectum nephrosclerosis (pyelonephritic?) fairly numerous plasma cells in the bone marrow

Case 67 Female born in 1891 Since 1925 she had periodically had joint and muscle pain In 1953 she was cared for because of pneumonia In 1954 her previously only moderate symptoms fatigue headache dizziness and generalized pain became more severe and during 2 periods she had attacks of marked dizziness nausea and weakness of the left leg At the turn of the year 1954—55 she was admitted to hospital for these symptoms cystitis and fever Physical examination revealed nothing remarkable Hb 9 3 g/100 ml R B C 2 8 mill W B C 3 400 and thrombocytes 180 000/mm³ E S R 90 mm/1 hr an M-component in the serum (γG 1 6 g/100 ml) pyuria bacteriuria X ray of the chest biliary tract colon kidneys and skeleton revealed no abnormalities Low grade fever gradually disappeared in association with antibiotic therapy She was admitted to hospital later in 1955 because of dizziness vomiting and fever but recovered in a few weeks In 1956 she again entered hospital this time because of fatigue and generalized pain Physical examination revealed nothing remarkable except tenderness of the thoracic cage She had no anaemia but leukopenia and the M-component was largely unchanged Sternal puncture showed 7 % plasma cells some large with loose chromatin and many with nucleoli Rheumatoid factor (sensitized sheep cell test) could not be demonstrated In 1958 she was admitted with the same picture Investigation showed nothing new the x ray appearance of the skeleton was normal the number of plasma cells in a smear poor in cells was 4 %, of which half were plasmacellular reticulum cells In 1959 the clinical picture was dominated not only by the usual symptoms but also by increasing forgetfulness and symptoms of cystitis The bone marrow was largely unchanged as was R B C and W B C From 1960 on she was cared for at an infirmary In December 1960 she had kerato-conjunctivitis sicca and the bromsulphalein clearance was slightly impaired In November 1961 she was admitted to hospital because of cystopyelitis which subsided Rheumatoid factors were now demonstrated in the serum The serum creatinine concentration was normal Treatment with melphalan had no demonstrable effect on her condition Skeletal x ray and sternal puncture in the autumn of 1962 produced no evidence of myelomatosis The concentration of the gamma fraction increased markedly during the last year (Fig 16 p 00) Her mental bluntness progressed the patient was confined to bed and occasionally had attacks of fever which were ascribed to bronchopneumonia and she died in July 1963 — Necropsy Pronounced arteriosclerosis diffuse

marrow electrophoretic pattern unchanged no proteinuria, no osteolytic lesions

Case 35 Female, born in 1900 In 1911 to 1941 she was repeatedly cared for because of osteo myelitis of the left femur and right foot In 1918 gastroenterostomy was done because of stenosing duodenal ulcer In 1937 she was cared for because of achylia gastrica cum anaemia secundaria and in 1943 for nephrolithiasis 1950 she was admitted because of anaemia fatigue and loss of hair B M R was —30% and thyroid substitution was started with a good effect In 1955 she was treated because of thrombophleb itis Hb 12 0 g/100 ml R B C 3 7 mill serum iron 41 µg/100 ml E S R 8 mm/1 hr Electro phoresis showed a largely normal pattern The Mantoux reaction was positive In 1958 the patient had herpes zoster In 1959 she had *erythema nodosum* roentgen examination showed lymphoma of the pulmonary hilus and the Mantoux reaction was negative R B C 3 8 mill E S R 27 mm/1 hr, electrophoretic pattern unchanged She afterwards felt well until April 1964 when she was admitted because of pharyng itis which soon healed R B C 3 6 mill E S R 59 mm/1 hr and a serum M component (γA 0 2 g/100 ml) In October the bone marrow was found to contain 1 % plasma cells and the con centration of the M component was 0 5 g/100 ml In April 1965 she was in a good general condition R B C 3 7 mill the bone marrow was un changed there was no proteinuria and skeletal x ray showed no lesions In September 1965 the concentration of the M component was 0 8 g/100 ml

Case 41 Male born in 1913 In May 1961 he developed fever up to 40 C but no other symp toms He afterwards felt tired but physical examination revealed nothing remarkable E S R 35 mm/1 hr in the serum an M component (γA 0 6 g/100 ml) the concentration of the gamma fraction 1 5 g/100 ml Because of persisting tiredness and headache he was admitted to hospital in July The clinical picture was unchanged E S R 14 mm/1 hr The peripheral blood showed as before eosinophilia (725/mm³) The patient remained tired and complained of headache In January 1962 he had an attack of grand mal and his relatives noticed progressive mental change with bluntness apathy and memory difficulties In May he was admitted to the department of psychiatry Neurological examination ophthalmoscopy air encephalo graphy and right sided carotid angiography revealed nothing remarkable E E G showed a right sided abnormality The cerebro spinal fluid contained a normal number of cells but the protein concentration was 180 mg/100 ml Electrophoresis showed the same picture as before After he had been home for some time he was again admitted in August because of deterioration including slurred speech The E E G showed progression with bilateral abnormality C S F protein had increased to 230 mg/100 ml Re investigation with air encephalography bilateral carotid and vertebral angiography revealed nothing remarkable Babinski s reaction later became positive and papilloedema appeared Extensive investigations including skeletal x ray muscle biopsy (poly arteritis nodosa?) culture of the C S F for yeasts and bacteria cytological and electro phoretic examination of the C S F electro phoresis of the urine serological studies for R S S E toxoplasmosis and listeriosis failed to reveal the cause of the symptoms Because of the M component and 6 % (later 16 %) atypical plasma cells in the bone marrow (Plate II 1 2) he was admitted to the medical department in January 1963 He was not properly oriented in time or space his gait was unsteady and atactic the knee and ankle jerks were brisk and Babinski s sign was positive E E G showed if anything an improvement compared with August 1962 The electrophoretic pattern was unchanged as it was at several controls the last in November 1965 Treatment with melphalan was accompanied by a slow but distinct improve ment He became more oriented and the papill oedema disappeared At follow up in 1964 he appeared to be psychically normal though his relatives thought that he was still changed psychically (forgetfulness ixoidia) his gait was atactic the tendon reflexes and Babinski s sign were still abnormal E E G was broadly speak ing normal and the total protein content of the C S F was 78 mg/100 ml In the spring of 1965 the clinical picture was unchanged The bone marrow contained 3 % plasma cells of the same appearance as before and he had no proteinuria

Case 63 Female born in 1895 During the years 1922 to 1948 she often had cystitis and sometimes pyelitis In 1948 cholecystectomy was done because of biliary symptoms for 3 years In July 1958 thrombophlebitis supervened In August she had a severe attack resembling the biliary symptoms she had had before with pain vomiting dark urine fever and diarrhoea She afterwards became short of breath she had a cough fever developed and roentgen examina tion showed right sided pneumonia R B C 3 5 mill 2 % plasma cells in the bone marrow an M component (γG 2 2 g/100 ml) in the serum no proteinuria She left hospital in a good general condition but occasionally had pain in the right hypochondrium and on one occasion she passed renal gravel In July 1959 she was admitted to hospital because of increasing tiredness and anorexia She had lost 8 kg since her previous spell in hospital R B C 3 4 mill in the bone marrow 3 % plasma cell The

concentration of the M-component had increased Roentgen examination of the stomach showed nothing remarkable Her condition was afterwards dominated by abdominal pain she deteriorated and anaemized The concentration of the M component increased to 3 9 g/100 ml (Fig 15 p 00) but at most 3 % plasma cells were noted in the bone marrow She died in August 1960 —Necropsy Large gastric cancer with metastases of the lymph nodes and infiltration of the right diaphragm plasma cell infiltration around the tumour and in the spleen lymph nodes and liver no bone marrow plasmocytosis concrements and pyelonephritic scars in right kidney

Case 61 Female born in 1887 Symptoms of gall stones without fever or jaundice started in 1950 In 1953 she was admitted because of pneumonia and in 1958 because of iridocyclitis The R B C was then 4 2 mill and the E S R 12 mm/1 hr In 1960 she was admitted for thrombosis of the lower leg R B C 4 1 mill in the bone marrow 11 % plasma cells with crystal shaped holes in the cytoplasm (Plate 1 I) and with nucleoli in about one fourth of the cell Serum creatinine 1 4 mg/100 ml the urine contained protein but not pus E S R 37 mm/1 hr in the serum an M component (γG 2 6 g/100 ml) X ray showed moderate osteoporosis As later slight glycosuria was occasionally observed but the blood sugar level was normal In 1961 she was admitted for examination to find out whether cytostatic treatment was indicated She was tired but otherwise her general condition was unchanged The blood values and the concentration of the M-component were largely unchanged The serum creatinine was 1 8 mg 100 ml and the urinary sediment was normal A light chain component was demonstrated in the urine as well as albumin and other globulin fraction The bone marrow contained 3 % plasma cells of the same type as before Cytostatic treatment was not considered indicated and the patient was sent home In the spring of 1963 an attack of cystitis was curtailed by sulpha treatment When admitted in April 1964 he had been thirsty and anorectic she had lost weight and had nocturia She was emaciated R B C 3 0 mill bone marrow and M-component unchanged serum creatinine 4 2 mg 100 ml serum calcium 8 2 and phosphorus 3 7 mg/100 ml no osteomalacia or osteolytic lesion Analysis of the urine because of suspected Fanconi syndrome showed amino acids Fluid therapy produced an improvement but cholecystitis supervened and operation was considered indicated despite her poor condition After the operation the patient occasionally had intestinal bleeding and 2 months later she died in July 1964 Necropsy Deep ulcerations particularly in the duodenum and together with unspecific inflammation also in the tongue oesophagus and rectum nephrosclerosis (pyelonephritic?) fairly numerous plasma cells in the bone marrow

Case 6? Female born in 1891 Since 1925 she had periodically had joint and muscle pain In 1953 she was cared for because of pneumonia In 1954 her previously only moderate symptoms fatigue headache dizziness and generalized pain became more severe and during 2 periods she had attacks of marked dizziness nausea and weakness of the left leg At the turn of the year 1954—55 she was admitted to hospital for these symptoms cystitis and fever Physical examination revealed nothing remarkable Hb 9 3 g/100 ml R B C 2 8 mill W B C 3 400 and thrombocytes 180 000/mm³ E S R 90 mm/1 hr an M-component in the serum (γG 1 6 g/100 ml) pyuria bacteriuria X ray of the chest biliary tract colon kidneys and skeleton revealed no abnormalities Low grade fever gradually disappeared in association with antibiotic therapy She was admitted to hospital later in 1955 because of dizziness vomiting and fever but recovered in a few weeks In 1956 she again entered hospital this time because of fatigue and generalized pain Physical examination revealed nothing remarkable except tenderness of the thoracic cage She had no anaemia but leukopenia and the M-component was largely unchanged Sternal puncture showed 7 % plasma cells some large with loose chromatin and many with nucleoli Rheumatoid factor (sensitised sheep cell test) could not be demonstrated In 1958 she was admitted with the same picture Investigation showed nothing new the x ray appearance of the skeleton was normal the number of plasma cells in a smear poor in cells was 4 % of which half were plasmacellular reticulum cells In 1959 the clinical picture was dominated not only by the usual symptoms but also by increasing forgetfulness and symptoms of cystitis The bone marrow was largely unchanged as was R B C and W B C From 1960 on she was cared for at an infirmary In December 1960 she had kerato-conjunctivitis sicca and the bromsulphalein clearance was slightly impaired In November 1961 she was admitted to hospital because of cystopyelitis which subsided Rheumatoid factors were now demonstrated in the serum The serum creatinine concentration was normal Treatment with melphalan had no demonstrable effect on her condition Skeletal x ray and sternal puncture in the autumn of 1962 produced no evidence of myelomatosis The concentration of the gamma fraction increased markedly during the last year (Fig 16 p 00) Her mental bluntness progressed the patient was confined to bed and occasionally had attacks of fever which were ascribed to bronchopneumonia and she died in July 1963 —Necropsy Pronounced arteriosclerosis diffuse

cerebral atrophy bronchiectasie focal pneumonia, cystopyelitis steatosis of the liver liver cirrhosis diffuse increase of plasma cells in the bone marrow and infiltration of plasma cells in the liver spleen kidneys and in enlarged lymph nodes from various sites

Case 68 Female born in 1887 In 1952 and *1960 she had had pain which was ascribed to* renal stone In 1961 x ray showed a calcification in a position corresponding to the lower left ureter In the autumn of 1964 she felt tired thirsty and nauseated she vomited occasionally and lost 5 kg in one month The patient had lumbar pain and occasionally had a bloody mucous discharge from the nose In January 1965 she was admitted to hospital Nothing remarkable was found on physical examination R B C 2 7 mill in the bone marrow 10 % *plasma cells half of which had nucleoli often* with a diameter of one fourth to one half of the nucleus The serum creatinine was 18 and the serum phosphorus 8 mg/100 ml F S R 11 mm/1 *hr The serum contained an M component* (γG 1 9 g/100 ml) and the urine a light chain component together with other protein fractions Three weeks later she died in oliguria and uraemia —Necropsy Diffuse plasma cell increase in the bone marrow but not sufficiently to justify a diagnosis of myelomatosis A papillary necrosis was seen in one of the kidneys and both kidneys showed lymphocyte infiltrated scars in the cortex dilatated tubules with eosinophilic cylinders and swollen glomerular tufts but no deposits of amyloid demonstrable in polarised light

Case 74 Male born in 1882 Gastroenterostomy was done in 1939 because of symptoms of peptic ulcer since 1925 In 1956 and 1957 an ulcer was again demonstrable and on the latter occasion the R B C was 3 4 mill and F S R 33 mm/1 hr Half a year later he was admitted because of abdominal pain x ray showed nothing remark*able R B C 3 9 mill E S R 36 mm/1* hr and the serum which had a total protein concentration of 5 5 g/100 ml contained an M component *(γA 0 5 g/100 ml) No proteinuria He was* discharged without further treatment In 1959 he sustained a fracture of the femoral neck During treatment he had haematemesis and melaena which ceased *spontaneously In 1960* he was treated for epigastric pain R B C 2 6 mill E S R 28 mm/1 hr Because of cerebral arteriosclerosis he was not referred for operation That year he received radiotherapy for basal cell cancer In April 1961 he was re admitted because of epigastric pain As before the stools contained blood R B C 3 2 mill 7 % mainly mature plasma cells in the bone marrow E S R 43 mm/1 hr M component concentration 0 9 g/100 ml (total protein 7 8 g/100 ml) in the urine a light chain component no osteolytic lesions

Half a year later the R B C was 4 3 mill the M component concentration 1 0 g/100 ml and the total protein concentration 8 3 g/100 ml One year later (October 1962) he sustained a fracture of the femoral neck and during treatment intestinal bleeding supervened and he died The E S R on that occasion was 41 mm/1 hr Electrophoresis was not done but the bone marrow contained 9 % plasma cell mainly mature and some in clusters surrounding a reticulum cell —Necropsy Large duodenal ulcer *responsible for the bleeding and ulcer in the* gastroenterostomy No plasma cell increase in the bone marrow in vertebrae femora rib or skull

Case 77 Female born in 1894 She had been largely healthy until April 1965 when nausea vomiting chills and widespread urticaria *supervened She had fever and tetracycline* was instituted Fever and urticaria subsided but vomiting became more frequent On admission to hospital in May she was in a bad general condition but otherwise physical examination was unrewarding R B C 3 3 mill W B C 26 000/mm³ 95 % of which were neutrophilic granulocytes thrombocytes 190 000 in the bone marrow 4 % plasma cell one third of which were plasmacellular reticulum cell Serum electrophoresis showed hypoalbuminaemia (2 3 g/100 ml) an increase of the alpha fractions and an M component (γG 1 0 g/100 ml) Proteinuria but no light chain component serum creatinine 4 8 mg/100 ml She became oliguric and died 3 weeks after admission —Necropsy Polyarteritis nodosa subacute glomerulonephritis diffuse myelomatosis

Case 106 Male born in 1887 He had had psoriasis since about 1910 and psoriatic arthropathy since about 1940 In the fourties he occasionally used analgetics containing amidopyrine In 1948 he was admitted to hospital because of dyspnoea and cardiosclerosis was diagnosed R B C 4 4 mill W B C 3 100 mm³ (46 % neutrophilic granulocytes) In 1950 he was *treated in hospital because of bloody* diarrhoea and x ray showed signs of ulcerative colitis R B C 3 4—2 9 mill W B C 3 300—1 500 thrombocytes 405 000/mm³ reticulocytes 0 8—2 % *active erythropoiesis in the* bone marrow Sulpha was started he improved and was afterwards treated mainly for psoriasis In 1954 R B C 2 7 mill W B C 1 500 thrombocytes *81 000—140 000 reticulocyte 2 % in the* bone marrow active erythropoiesis and 3 % plasma cells serum iron 165 μg/100 ml Coomb s test negative In 1956 he was admitted because of *dyspnea, and remittent fever He was in a* bad general condition R B C 1 2 mill W B C 1 300 (40 % neutrophilic granulocytes) thrombocytes 52 000 in the bone marrow 3 % plasma cell

38 % lymphocytes and act ve erythropo e s Serum electrophores s revealed an M component (0 % g/100 ml) gamma fraction 0 9 g/100 ml No prote nur a D arrl oea and card ac in compensat on supervene l and he d ed 4 months after adm ss on — Necropsy Myelomato s act ve erythropo e s sparse myelop o es s ulcer at ve col t s

MYELOMATOSIS GROUP

Case 40m Male l orn n 1898 After having had pa n in the left h p for 2 years l e sought med cal adv ce H s general cond t on was good R B C 4 7 m ll F S R 15 mm/1 hr an M component (γG 0 7 g 100 ml) was found in the serum l ut tl ere was no prote nuria X ray osteolytic le ion of tl e 4th lumbar vertel ra a rarefact on the s ze of the end of the tl umb over the left acetal ulum and another about 8 mm in d ameter in the sku ll No pr mary tumour could be found and puncture of the sternum and sp nous process of a lumbar vertebra produced no evidence of myelomato s The pat ent was sent home in February 1961 and requested to return for follow up On supervent on of back pa n however he f rst went to a therapeut st and was g ven phys o therapy Not unt l September was he ad n tted to hosp tal an l tl en l ecause of ncreas ng pa n He had then lost we ght (from 80 to 65 kg) the concentrat on of the M component had increa ed to 3 1 g 100 ml the bone marrow conta ned 5 % very atyp al pla ma cells and x ray showed w de pread osteolyt c les ons He responded to melphalan tl erapy

Case 47m Female born in 1895 In January 1957 she compla ned of pa n of the left h p extend ng down to the calf and weakness of dorsal flex on of the foot wa found F S R 84— 97 mm 1 hr Hb 13 9 g 100 ml She refused ho p tal sat on In August 1958 she ret rned l ecau e of low back pa n wh ch sub ded Hb 13 0 g 100 ml She refused hosp tal sat on for nve t gat n of F S R of 84 114 mm 1 hr In August 1962 she ha l a cold and then consented to adm on Apart from pa n n the r ght shoul ler wh ch she had had for some years she felt well and phys cal exam nat on showe l noth ng remarkal le Hb 12 8 g 100 *ml* R B C 3 8 m ll in the l one marrow 11 % *ma nly* normal plasma cells one fourth of wh ch however had ma nly small n cl ol F S R from now on ranged l etwee 120 130 mm 1 hr Tl e serum conta ned an M component (G 2 8 g 100 ml) a d the all n n concentrat on was 3 3 g 100 ml The ur ne conta ned no l ght cl a n *component* and x ray of the skeleton wa *normal unt* l Octol er 1964 when vertel ral *comp ress on* appeared The M component concentrat on rose (f g 13 p 00) but he felt well and kept her we ght In November 1963 the R B C was 3 3 and in March 1964 3 0 m ll Sl e had then for some months felt tired and d zzy and for the last 2 weeks she had had attacks of nose l leed ng and tl e M component concentration was 5 3 g/100 ml She was afterwards treate l ma nly w th melphalan

Case 55m Female born in 1906 A si ter had dissen nated lupus and another had an F S R of 30—40 mm/1 hr and occasional jo nt pain The pat er t had s nce ch ldhood often had infect ons of the throat with associate l joint pa n In 1938 tons llectomy was done w thout any effect on the incidence of tl ese infect ons In 1941 she was treated for several months l ecause of fever and jo nt pa n after a common cold In 1946 she was adm tted to hosp tal l ecause of joint pain without ol ject ve f nd ngs except an F S R of 30 40 mm 1 hr In 1948 sl e was adm tted l ecau e of lobar pneumonia and later that year because of jo nt pain and ep odes of urt car a The F S R was uncl anged Roentgen exam nat on showed irregi lar haustra n tl e de cend ng and in the s gmo d colon In 1951 she compla ned for the f rst t me of pa n n the left half of the al domen R B C 4 5 m ll E S R 38 mm/1 hr In 19 6 curettage was done beca se of vag nal d scharge and dyspareunia Palpation revealed a lump the s ze of a goose s egg to the left and beh d the uterus wh ch was d placed to the r ght X ray of the colon showe l d vert culos s At exam nat on for al dom nal pa n in 1957 the R B C was 3 6 mill F S R 40 mm/1 hr Bence Jones test was negat ve an l an M-component was found n the serum (γG 1 6 g 100 ml) In 1958 she was admitted for ext rpa t on of the affected part of tl e colon She had l l ary symptoms and cholecy *tectomy* was performed f st R B C 4 3 mill n the l one marrow 11 % ma nly mature plasma cells the M component concentrat on was unchanged *and* x ray showe l sl ght osteoporos s of the *pelvis* and of the luml ar sp ne After the *operat on* she had herpes zoster In Decemb er 19 9 the s gmo d colon was resected Patholog st s rep ort d vert cu l t s w th inf ltrat *on ma nly of eos no* ph l c *leukocytes and plasma cells* The concentra tion of the M-*component had now r en* to 2 5 *g 100 ml Her* cond t on was afterwar ls un change l but n March 1961 the concentration of the M component was 4 9 and in Octol er 6 5 *g 100 ml* She was then adm tted becau e of l lee l ng from the nasal and tl e oral muco a R B C 3 4 *m ll* 22 % mature pla ma cells in the *bone marrow* (*Plate II 3 6*) E S R 121 mm l hr X ray showed compress on of a vertebra Treatment w th melphalan was tarted Her co l tion was afterwards character ed l y *repeated* nfect ons loss of bodywe ght and gen *eral leterioration* n desp te a lecrea e in concentra *t on of the* M component In Novemb er 1962 she ha l severe al dominal pa n and laparotomy

cerebral atrophy, bronchiectasies focal pneumonia cystopyelitis steatosis of the liver liver cirrhosis diffuse increase of plasma cells in the bone marrow and infiltration of plasma cells in the liver spleen kidneys and in enlarged lymph nodes from various sites

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chromatin and one or more small nucleoli E S R 47 mm/1 hr M-component 3 4 g/100 ml and no light chain component in the urine Treatment with melphalan was started and after 2 months the patient was sent home She could then walk with a stick The concentration of the M-component fell to about 2 5 g/100 ml and apart from 2 episodes of bronchopneumonia she managed well

Case 64m Female born in 1889 The patient had been cared for in 1924 because of chronic salpingo-oophoritis In 1932 she was subjected to amputation of the myomatous uterus and bilateral salpingo oophorectomy Diabetes was discovered in 1949 and was treated dietetically In 1955 she was admitted because of acute cholecystitis with pancreatitis and in March 1956 because of fatigue shortness of breath palpitation stabbing precordial pain dizziness and headache She had by then lost weight from 96 to 62 kg in 7 years Cardiosclerosis was diagnosed R B C 4 9 mill E S R 22 mm/1 hr a serum M component (γG 0 7 g/100 ml) no proteinuria She afterwards did fairly well until 1957 when she was cared for at an infirmary for 2 months because of dizziness Hb 14 2 g/100 ml E S R 20 mm/1 hr In 1958 she had a relapse of cholecystitis and again in 1959 in which year he was admitted because of acute otitis In March and August 1961 she was cared for because of gangrene of the large toe E S R 31 mm/1 hr In September that year he sustained a fracture of the femoral neck and after a spell in hospital she was admitted to an infirmary from where she was sent home in September 1962 During 1962 her bodyweight was constant R B C 4 7 mill and the E S R 43 mm/1 hr In February 1963 she was admitted after a few days increasing confusion and generalized pain R B C 3 0 mill in the bone marrow 12 % plasma cells most of them normal but some with central nuclei of atypical shape (hourglass kidney shaped) with a fine chromatin sometimes with nucleoli and surrounded by a narrow margin of cytoplasm E S R 129 mm/1 hr M component 6 4 g/100 ml The urine contained no light chain component Skeletal x ray could not be done before the woman died 3 weeks after admission —Necropsy Myelomatosis haemorrhagic fibrinous pericarditis grave general arteriosclerosis fibrously healed myocardial infarction

Case 71m Male born in 1905 In 1953 he complained of pain due to a renal stone which was passed spontaneously From December 1954 he had paraesthesia anaesthesia and weakness of the feet and legs He was admitted in February 1955 with signs of impaired superficial and deep sensibility and loss of knee and ankle jerk The protein content of the C S F was 162 mg/100 ml Electrophoresis of the C S F revealed an M component with the same rate of migration and relative concentration as the component in the serum (γG 0 3 g/100 ml) R B C 5 2 mill in the bone marrow 3 % plasma cells mainly normal but sometimes large with pale shedding cytoplasm and coarse but loose chromatin E S R 2 mm/1 hr no proteinuria X ray of the skull was unrewarding After 4 weeks treatment with ACTH (30 U/day) the patient was sent home in unchanged condition The symptoms progressed and gradually involved also the arm In March 1956 the patient was again admitted and was now confined to bed The sternal marrow contained 7 % plasma cells and biopsy of the iliac crest showed myelomatosis X ray of the skull still showed nothing abnormal but sclerotic patches were seen in the spine and in the pelvis From April to August prednisone (20—25 mg/day) was given The patient did not become anaemic the concentration of the M component remained constant but the neurological symptoms progressed He deteriorated and died in October 1956 —Necropsy Myelomatosis osteosclerotic foci with coarse trabeculae surrounded by lacunae filled with plasma cells

Case 72m Male born in 1894 (case E.M in Waldenstrom 1964 a) Apart from a previous traumatic injury and pneumonia the man had always felt healthy until June 1959 when he was admitted to hospital because of roentgenologically verified duodenal ulcer R B C 4 7 mill 4 % mature plasma cells in bone marrow E S R 48 mm/1 hr in the serum an M component (γG 0 3 g/100 ml) Skeletal x ray showed a normal appearance apart from a few changes in the skull which at later examination were found to persist unchanged and probably were not manifestations of myelomatosis The patient left hospital in a good condition In the summer of 1960 he felt tired and in November that year he was admitted with anaemia and melaena which spontaneously ceased In December R B C was 4 2 mill (after blood transfusion) bone marrow unchanged E S R 15 mm/1 hr He was discharged in a good condition In May 1962 he had herpes zoster below the umbilicus Roentgen examination showed compression of the 11th thoracic vertebra and the 5th lumbar vertebra as well as osteoporosis and he was admitted to hospital in July The R B C the serum electrophoretic pattern and the number of bone marrow plasma cells were as before but 25 % of the cells had nucleoli compared with 13 % at the previous examination The pain subsided and he was discharged In September he had symptoms of gastritis and roentgen examination showed duodenal ulcer for which he was admitted to hospital in October He had by then lost weight (57—54 kg) and roentgen examination showed compression of the upper

showed a tumour at the junction between the colon and the rectum and extending down to the small pelvis The patient died in December —Necropsy Myelomatosis with plasma cell infiltration also in kidneys retroperitoneal fatty tissue and fatty tissue in the pelvis causing bilateral hydronephrosis and venous compression

Case 56m Male born in 1897 He was admitted in 1957 because of dizziness and R B C 5 0 mill and because of suspected polycytaemia No support for this diagnosis was produced Serum electrophoresis showed a normal pattern (gamma fraction 0 8 g/100 ml) In 1960 he was admitted because of back pain X ray showed a walnut sized lesion in the 12th thoracic vertebra which was compressed in the left transverse process of the vertebra and nearby part of the 12th rib R B C 3 3 mill 4 % plasma cells in the sternal marrow the serum contained an M component (γG 1 5 g/100 ml) and the concentration of the gamma fraction was 0 3 g/100 ml During roentgen treatment the concentration of the M component decreased and when the patient was admitted one year later it was 0 4 g/100 ml The R B C was then 4 8 mill The reason for admission was a cerebral vascular lesion which proved fatal —Necropsy at which special attention was focused on the roentgenologically changed area produced no evidence of myelomatosis

Case 58m Female born in 1891 In 1923 she underwent nephrectomy because of right sided tuberculosis In 1937 and 1940 she was operated upon for left sided, cystic mastopathy classified by the pathologist as precancerous' In 1943 she began to complain of low back pain with periodic exacerbations X ray in 1949 showed spondylosis Between 1945 and 1949 the E S R varied between 14 and 65 and was usually about 30 mm/1 hr In 1954 she was operated upon for cancer in the scar after mastectomy The E S R was continuously elevated (50—70 mm/1 hr) In May 1958 she was admitted because the serum had been found to contain an M component (γM 1 1 g/100 ml) She complained of fatigue for the last 4—5 years Physical examination revealed nothing remarkable R B C 4 4 mill in the bone marrow 12 % mainly normal plasma cells 6 % lymphocytes and no mast cells E S R 89 mm/1 hr no osteolytic lesion She was sent home but was re admitted in January 1959 because of increasing fatigue and pain in the thoracic spine The results of examination were the same as before but roentgen examination showed patchy decalcification of the ribs She was sent home in unchanged condition In November she was re admitted because of increasing pain in the back and chest R B C 4 2 mill the concentration of the M component was 0 7 g/100 ml X ray showed widespread unequivocal osteolytic lesions She was treated with urethan (120 g) without effect on the pain or on the concentration of the M component In July 1960 she was admitted because of increasing pain that could be explained by compression of a thoracic vertebra She was treated with melphalan The concentration of the M component fell to 0 3 g/100 ml The patient was afterwards treated at the outpatient department, she complained of pain but apart from the abnormal curvature of the spine due to vertebral compressions she was in a relatively good general condition In 1962 electrophoresis of the urine showed a light chain component In January 1963 a pleural effusion was found to contain cancer like cells R B C 3 2 mill the M component was unchanged Melphalan treatment was continued In January 1964 the concentration of the M component was 0 4 g/100 ml In March she was admitted mainly because of shortness of breath and was found to have abundant pleural exudate and densities in the pleural parenchyma She deteriorated successively In May the concentration of the M component was 1 4 and in June 1 6 g/100 ml X ray showed progression of the skeletal lesions and in August the patient died —Necropsy Cancer with metastases in scar after mastectomy in lungs liver spleen lymph nodes but not in the skeleton myelomatosis

Cases 59m Female born in 1889 The patient had herpes zoster corresponding to the XII th spinal segment in 1957 Later that year she was admitted because of right sided abdominal pain Hb 11 2 g/100 ml R B C 3 7 mill The E S R fell from 68 to 25 mm/1 hr Appendiceal abscess was suspected but laparotomy revealed a normal appendix In 1958 the patient was admitted because of cholecystitis with jaundice Cholecystectomy was done Hb 13 1 g/100 ml the lowest E S R noted was 27 mm/1 hr in the serum an M component (γG, 1 1 g/100 ml) She afterwards felt well until spring 1962 when she had a short spell of pain in the entire body In April 1962 she sustained a fracture of the femoral neck and was operated upon with a good result Hb 12 6 g/100 ml and the lowest E S R noted was 27 mm/1 hr X ray showed moderate osteoporosis of the lumbar spine but no vertebral compressions In December 1962 she complained of increasing low back pain X ray in January 1963 showed distinct progression of osteoporosis and compression of several vertebrae She deteriorated successively lost 10 kg body weight in 3 months and was confined to bed because of pain Repeated x ray showed further progression but no osteolytic lesions and she was admitted to hospital Hb 11 5 g/100 ml R B C 3 4 mill in the bone marrow 10 % plasma cells mostly mature but a few with pale structureless or fine

chromatin and one or more small nucleoli E.S.R. 47 mm/1 hr. M-component 3.4 g/100 ml and no light chain component in the urine. Treatment with melphalan was started and after 2 months the patient was sent home. She could then walk with a stick. The concentration of the M-component fell to about 2.5 g/100 ml and apart from 2 episodes of bronchopneumonia she managed well.

Case 61m. Female, born in 1889. The patient had been cared for in 1924 because of chronic salpingo-oophoritis. In 1932 she was subjected to amputation of the myomatous uterus and bilateral salpingo-oophorectomy. Diabetes was discovered in 1949 and was treated dietetically. In 1955 she was admitted because of acute cholecystitis with pancreatitis and in March 1956 because of fatigue, shortness of breath, palpitation, stabbing precordial pain, dizziness and headache. She had by then lost weight from 96 to 62 kg in 7 years. Cardiosclerosis was diagnosed. R.B.C. 4.9 mill. E.S.R. 22 mm/1 hr, a serum M component (γG, 0.7 g/100 ml), no proteinuria. She afterwards did fairly well until 1957 when she was cared for at an infirmary for 2 months because of dizziness. Hb 14.2 g/100 ml. E.S.R. 20 mm/1 hr. In 1958 she had a relapse of cholecystitis and again in 1959, in which year she was admitted because of acute otitis. In March and August 1961 she was cared for because of gangrene of the large toe. E.S.R. 31 mm/1 hr. In September that year she sustained a fracture of the femoral neck and after a spell in hospital she was admitted to an infirmary from where she was sent home in September 1962. During 1962 her bodyweight was constant. R.B.C. 4.7 mill. and the E.S.R. 43 mm/1 hr. In February 1963 she was admitted after a few days increasing confusion and generalized pain. R.B.C. 3.0 mill.; in the bone marrow 12 % plasma cells, most of them normal but some with central nuclei of atypical shape (hourglass, kidney-shaped) with a fine chromatin, sometimes with nucleoli and surrounded by a narrow margin of cytoplasm. E.S.R. 129 mm/1 hr. M component 6.4 g/100 ml. The urine contained no light chain component. Skeletal x-ray could not be done before the woman died 3 weeks after admission.—Necropsy: Myelomatosis, haemorrhagic fibrinous pericarditis, grave general arteriosclerosis, fibrously healed myocardial infarctions.

Case 71m. Male, born in 1905. In 1953 he complained of pain due to a renal stone which was passed spontaneously. From December 1954 he had paraesthesia, anaesthesia and weakness of the feet and legs. He was admitted in February 1955 with signs of impaired superficial and deep sensibility and loss of knee and ankle jerks. The protein content of the C.S.F. was 162 mg/100 ml. Electrophoresis of the C.S.F. revealed an M component with the same rate of migration and relative concentration as the component in the serum (γG, 0.3 g/100 ml). R.B.C. 5.2 mill.; in the bone marrow 3 % plasma cells, mainly normal but sometimes large with pale, bedding cytoplasm and coarse but loose chromatin. E.S.R. 2 mm/1 hr, no proteinuria. X-ray of the skull was unrewarding. After 4 weeks treatment with ACTH (30 U/day) the patient was sent home in unchanged condition. The symptoms progressed and gradually involved also the arms. In March 1956 the patient was again admitted and was now confined to bed. The sternal marrow contained 7 % plasma cells and biopsy of the iliac crest showed myelomatosis. X-ray of the skull still showed nothing abnormal, but sclerotic patches were seen in the spine and in the pelvis. From April to August prednisone (20—25 mg/day) was given. The patient did not become anaemic, the concentration of the M component remained constant but the neurological symptoms progressed. He deteriorated and died in October 1956.—Necropsy: Myelomatosis, osteosclerotic foci with coarse trabeculae surrounded by lacunae filled with plasma cells.

Case 72m. Male, born in 1894 (case E.M. in Waldenström 1964 a). Apart from a previous traumatic injury and pneumonia the man had always felt healthy until June 1959 when he was admitted to hospital because of roentgenologically verified duodenal ulcer. R.B.C. 4.7 mill.; 4 % mature plasma cells in bone marrow. E.S.R. 48 mm/1 hr; in the serum an M component (γG, 0.3 g/100 ml). Skeletal x-ray showed a normal appearance apart from a few changes in the skull, which at later examination were found to persist unchanged and probably were not manifestations of myelomatosis. The patient left hospital in a good condition. In the summer of 1960 he felt tired and in November that year he was admitted with anaemia and melaena, which spontaneously ceased. In December R.B.C. was 4.2 mill. (after blood transfusions), bone marrow unchanged. E.S.R. 15 mm/1 hr. He was discharged in a good condition. In May 1962 he had herpes zoster below the umbilicus. Roentgen examination showed compression of the 11th thoracic vertebra and the 5th lumbar vertebra as well as osteoporosis, and he was admitted to hospital in July. The R.B.C., the serum electrophoretic pattern and the number of bone marrow plasma cells were as before, but 25 % of the cells had nucleoli compared with 13 % at the previous examination. The pain subsided and he was discharged. In September he had symptoms of gastritis and roentgen examination showed duodenal ulcer, for which he was admitted to hospital in October. He had by then lost weight (57—54 kg) and roentgen examination showed compression of the upper

end plate of the first lumbar vertebra but the R B C and the M component concentration were unchanged In February 1964 he was admitted because of pain in the left flank Since he had left hospital he had felt tired and complained of symptoms of ulcer His general condition was as before apart from tenderness to percussion between the thoracic and lumbar spine R B C 4 2 mill in the bone marrow 17 % plasma cells 66 % of which had nucleoli M component 1 1 g/100 ml no proteinuria Skeletal x ray showed no progress He was treated with melphalan and the pain abated In May he left hospital In August he was admitted because of pain in the chest and spine for one week R B C 3 6 mill M component 1 7 g/100 ml further vertebral compressions and osteolytic foci in the ribs Serum calcium 7 5 mEq/l He deteriorated rapidly and died in pulmonary oedema —Necropsy Myelomatosis myocardial fibrosis with cardiac incompensation left sided pyelitis

Case 78m Female born in 1903 She had had a carpal tunnel syndrome since 1958 and was admitted in 1962 for investigation of this syndrome and periocular haemorrhages Examination showed R B C 3 5 mill 10 % plasma cells in the bone marrow gamma fraction 0 6 g/100 ml no serum M component but a light chain component in the urine and osteolytic lesions of the skull Large amyloid masses were removed from the carpal region bilaterally Melphalan was given without any definite effect on the condition Later cardiac incompensation and renal insufficiency supervened and she died in 1963 —Necropsy showed widespread pericollagenous amyloidosis

SUBJECTS WITH γM COMPONENTS IN CHAPTERS IV AND VI

Case I Female born in 1885 The patient felt well until 1953 when symptoms of glaucoma supervened The E S R was 75 mm/1 hr In March 1960 the patient was admitted to hospital after 6 months fatigue anorexia and loss of body weight There was no increased bleeding tendency then or later Physical examination revealed nothing remarkable Hb 12 5 g/100 ml R B C 3 6 mill No blood was demonstrable in the stools The bone marrow contained 0 4 % plasma cells a lightly increased number of mast cells and 27 % lymphocytes including 38 % with nucleoli The E S R was 62 mm/1 hr and an M component (1 2 g/100 ml) was demonstrated in the serum but no light chain component in the urine Roentgen examination of the skeleton colon and stomach showed nothing remarkable The woman improved spontaneously and did well until the end of 1962 when she was again troubled by severe fatigue On admission her condition was unchanged as was the haematological picture M component 1 4 g/100 ml The bone marrow contained 73 % lymphocytes 0 4 % plasma cells and numerous mast cells On account of the anaemia determinations were made of the serum bilirubin reticulocytes serum iron total iron binding capacity haptoglobin carboxyhaemoglobin and cold agglutinin titre None of these tests showed anything remarkable There was no blood in the stools Coomb s test was normal as was later determination of vitamin B_1 and folic acid in the serum The patient was sent home improved without having received any special treatment She experienced a new spell of fatigue in August 1963 but afterward she felt fairly well In January 1965 she was re examined and then reported that she felt tired and short of breath Palpation revealed no hyperplasia of the lymph nodes spleen or liver R B C 2 8 mill in the bone marrow 93 % lymphocytes and the concentration of the M component 1 3 g/100 ml A trial with prednisone in June 1965 when the R B C was 2 6 mill was followed by an increase in the R B C to 3 4 mill and subjective improvement

Case II Female born in 1873 In 1950 she was admitted to an infirmary—for what was believed to be cholecystitis—with fever jaundice and Hb of 7 2 g/100 ml She was sent home after 2 months Apart from a few similar attacks she felt well until 1959 when she was admitted with jaundice She was otherwise in a good general condition R B C 1 1 mill reticulocytes 150 000/mm³ serum bilirubin 3 1 mg/100 ml haptoglobin 0 and carboxyhaemoglobin increased The serum contained cold agglutinins (some times $> 2 \times 10^6$) and an M component (0 8 g/100 ml) In the bone marrow numerous normoblasts some lymphocytes with loose chromatin and an increased number of mast cells Prednisone was given for a few months the patient felt better and the blood picture improved The R B C afterwards varied between 3 and 4 mill On repeated electrophoresis and examination of the bone marrow in the spring of 1965 the picture was unchanged

Case XII Male born in 1899 He had been largely healthy until January 9 1962 when he was admitted to hospital He had since December felt pulsating headache dizziness thirst shortness of breath and vomited a few times His bodyweight was unchanged Routine physical and neurological examination revealed nothing abnormal apart from somnolence Ophthalmoscopic examination showed beaded congested veins scattered haemorrhages and exudate Hb 8 0 g/100 ml R B C 2 8 mill W B C 9 500 including one fifth of atypical cells of the same type as in the bone marrow reticulocytes 32 000 and thrombocyte 300 000/mm³ The bone

marrow contained 35 % atypical cells (Plate III 6 7 and p 00) Serum electrophoresis showed no M component Urine electrophoresis however showed a general protein leakage and an M-component Plasma electrophoresis was done on sample taken in a tube with epsilon amino caproic acid which was used for collection of samples for coagulation studies and was found to prevent the gel formation An M component (6 7 g 100 ml) with the same rate of migration as in the urine was then demonstrated The E.E.G was severely abnormal with irregular theta-delta activity The clinical picture was dominated by somnolence He was treated with plasmapheresis Rheomacrodex® and melphalan without any effect On January 16 the patient was cold and clammy with B P 90 mm Hg he gradually deteriorated and died on January 25 —Necropsy Ruptured aneurysm in a branch of the superior mesenteric artery no signs of inflammation or amyloidosis in the vessel wall abundant atypical plasma cells in the bone marrow slight myeloma nephrosis in the basal ganglia in the brain small foci with necrosis of ganglion cells

Case XI Male born in 1886 In 1944 he was admitted to hospital because of haematuria of unknown origin The condition regressed spontaneously In 1945 he was again admitted for accentuation of abdominal pain which he had had for 5—6 years A gastric ulcer and gall stones were found In 1948 he was admitted for symptoms of cystitis and haematuria There was prostatic hyperplasia the urinary sediment contained numerous red and white blood cells cystoscopy showed haemorrhagic cystitis and urography revealed nothing abnormal The E S R was 19 mm 1 hr and he was not anaemic Because of repeated biliary attacks cholecystectomy was done The following years he felt fairly well but attended his doctor on a few occasions for symptoms which were conceived as signs of cholangitis and fluctuating increase of the E S R was noted (Fig 24 p 00) In August 1954 the patient noticed numbness of the heels and gradually pain in the hands and feet In March 1955 when he was admitted to hospital because of these symptoms and because of a high E S R he had had symptoms of cystitis for a few days For several years he had bled readily from the gingiva Examination showed parodontosis and a diffusely outlined decreased sensibility in the hands and feet The ocular fundi were normal Hb 10 1 g 100 ml R B C 3 2 mill reticulocytes 1 % In the bone marrow 52 % lymphocytes with loose chromatin and in 42 % nucleoli 0 3 % plasma cells but no mast cells The E S R was 131 mm 1 hr and the serum electrophoresis showed an M component (3 9 g 100 ml) Electrophoresis of the urine showed a light-chain component The urine contained numerous white blood cells N P N was normal Plasmapheresis was done with a good effect on the pain and stiffness of the fingers and decrease of the concentration of the M component to 2 1 g 100 ml The symptoms gradually recurred the M component increased and the anaemia progressed When he was admitted in April 1956 he had in one year lost 9 kg He had severe pain in the hands a symptom which disappeared on cessation with prednisone therapy He had moderate generalized purpura the lymph nodes spleen and liver were not enlarged R B C 2 8 mill M component 5 3 g 100 ml One month after admission he suddenly became worse with abdominal pain vomiting symptoms of cystitis gross haematuria increasing N P N and the patient died in pulmonary oedema —Necropsy Bronchopneumonia haemorrhagic cystopyelitis pronounced diffuse lymphatic infiltration in the lymph nodes spleen and the enlarged liver (1970 g) in which the infiltration had also produced a fairly well-defined orange sized mass routine histological examination of the lung showed an early cancer

Case XII Female born in 1871 In 1936 she received radium therapy for uterine cancer which afterwards produced no local symptoms At the end of the 20s she was troubled by pain stiffness and swelling of the distal interphalangeal joints in both hands and in the beginning of the 40s by pain and swelling of the metacarpophalangeal joints bilaterally In 1946 the E.S R was more than 100 mm 1 hr In 1948 she had pain and stiffness of the back and since the E.S R was high she was admitted to hospital Physical examination revealed nothing remarkable except stiffness of the back moderate swelling of the second metacarpophalangeal joint bilaterally and Heberden's nodules R B C 4 2 mill E.S R 103 mm 1 hr serum albumin 4 1 and globulin 2 9 g 100 ml Afterwards her condition was unchanged In February 1955 electrophoresis was done for the first time as a routine control and then an M-component of 1 6 g 100 ml was found In 1958 she sought advice for pain and numbness of the thigh a symptom which was judged as being due to spondylosis She had lost 6 kg during the last 2 years Besides signs of rheumatoid arthritis in the fingers and Heberden's nodules her status was normal Ophthalmoscopy revealed nothing remarkable but the conjunctival vessels showed granular flow R B C 2 8 mill in the bone marrow 40 % lymphocytes including many with loose chromatin 48 % with nucleoli and often raggy cytoplasm 1 % plasma cells and numerous mast cells No blood could be demonstrated in the stools the concentration of serum iron total iron binding capacity and bilirubin were normal Haptoglobin was increased (350 mg 100 ml) and there was a parallel increase of the concentration of the alpha and

end plate of the first lumbar vertebra but the R B C and the M component concentration were unchanged In February 1961 he was admitted because of pain in the left flank Since he had left hospital he had felt tired and complained of symptoms of ulcer His general condition was as before apart from tenderness to percussion between the thoracic and lumbar spine R B C 4 2 mill in the bone marrow 17 % plasma cells 66 % of which had nucleoli M component 1 1 g/100 ml no proteinuria Skeletal x ray showed no progress He was treated with melphalan and the pain abated In May he left hospital In August he was admitted because of pain in the chest and spine for one week R B C 3 6 mill M component 1 7 g/100 ml further vertebral compressions and osteolytic foci in the ribs Serum calcium 7 5 mEq/l He deteriorated rapidly and died in pulmonary oedema —Necropsy Myelomatosis myocardial fibrosis with cardiac incompensation left sided pyelitis

Case 78m Female born in 1903 She had had a carpal tunnel syndrome since 1958 and was admitted in 1962 for investigation of this syndrome and periocular haemorrhages Examination showed R B C 3 5 mill 10 % plasma cells in the bone marrow gamma fraction 0 6 g/100 ml no serum M component but a light chain component in the urine and osteolytic lesions of the skull Large amyloid masses were removed from the carpal region bilaterally Melphalan was given without any definite effect on the condition Later cardiac incompensation and renal insufficiency supervened and she died in 1963 —Necropsy showed widespread peri collagenous amyloido is

SUBJECTS WITH M COMPONENTS IN CHAPTERS IV AND VI

Case I Female born in 1885 The patient felt well until 1953 when symptoms of glaucoma *supervened The E S R was 75 mm/1 hr In* March 1960 the patient was admitted to hospital *after 6 months fatigue anorexia and loss of* body weight There was no increased bleeding tendency then or later Physical examination revealed nothing remarkable Hb 12 5 g/100 ml *R B C 3 6 mill No blood was demonstrable in* the stools The bone marrow contained 0 4 % plasma cells a slightly increased number of mast cells and 27 % lymphocytes including 38 % *with nucleoli The E S R was 62 mm/1 hr and* an M component (1 2 g/100 ml) was demonstrated in the serum but no light chain component in the urine Roentgen examination of the skeleton *colon and stomach showed nothing remarkable* The woman improved spontaneou ly and did well until the end of 1962 when she was again troubled by evere fatigue On admi ion her condition was unchanged as was the haematological picture M component 1 4 g/100 ml The bone marrow contained 73 % lymphocyte 0 4 % plasma cells and numerous mast cells On account of the anaemia determination were made of the serum bilirubin reticulocytes serum iron total iron binding capacity haptoglobin carboxyhaemoglobin and cold agglutinin titre None of these tests showed anything remarkable There was no blood in the stools Coomb s test was normal as was later determination of vitamin B_{12} and folic acid in the serum The patient wa sent home improved without having received any special treatment She experienced a new spell of fatigue in August 1963 but afterwards she felt fairly well In January 1965 she was re examined and then reported that she felt tired and short of breath Palpation revealed no hyperplasia of the lymph nodes spleen or liver R B C 2 8 mill in the bone marrow 93 % lymphocytes and the concentration of the M component 1 3 g/100 ml A trial with predni sone in June 1965 when the R B C was 2 6 mill was followed by an increase in the R B C to 3 4 mill and subjective improvement

Case II Female born in *1873 In 1950* she wa admitted to an infirmary—for what was believed to be cholecystitis—with fever jaundice and Hb of 7 2 g/100 ml She was sent home after 2 months Apart from a few similar attacks she felt well until 1959 when she was admitted with jaundice She was otherwise in a good general condition R B C 1 1 mill reticulocytes 150 000/mm³ serum bilirubin 3 1 mg/100 ml haptoglobin 0 and carboxyhaemoglobin increa ed The serum contained cold agglutinins (some times $> 2 \times 10^6$) and an M component (0 8 g/100 ml) *In the bone marrow numerou* normoblasts some lymphocytes with loo e chromatin and an increased number of mast cells Prednisone was given for a few month the patient felt better and *the blood picture im* proved The R B C afterwards varied between *3 and 4 mill On repeated electrophoresi and* examination of the bone marrow in the spring of 1965 the picture was unchanged

Case VII Male born in 1899 He had been largely healthy until January 9 1962 when he was admitted to hospital He had since December felt pul ating headache dizziness thirst short ness of breath and vomited a few time His bodyweight was unchanged Routine phy ical and neurological examination revealed nothing abnormal apart from somnolence Ophthalmo scopic examination showed beaded conge ted veins scattered haemorrhages and exudate Hb 8 0 g/100 ml R B C 2 8 mill W B C 9 500 including one fifth of atypical cells of the same type as in the bone marrow reticulocytes 32 000 and thrombocytes 300 000/mm³ The bone

(12 % lymphocytes) In 1959 the patient was admitted mainly because of shortness of breath and rapid loss of weight Physical examination revealed nothing remarkable R B C 1 9 mill W B C 5 500 of which 22 % were neutrophilic leucocytes and 75 % lymphocytes the thrombocytes decreased rapidly from 114 000 to 16 000/mm³ in the bone marrow 91 % normal lymphocytes and an increased number of mast cells The serum contained an M-component (γG 0 7 g/100 ml) The patient was sent home after blood transfusion A few months later the spleen was palpated just below the costal arch From April 1959 until September 1960 he was admitted on 8 occasions The clinical picture was unchanged and was dominated by anaemia which made transfusions necessary There were signs of hyperhaemolysis (sometimes slightly increased serum bilirubin occasionally more than 50 000/mm³ of reticulocyte decreased concentration of haptoglobin and increased carboxyhaemoglobin Coombs test negative) In November 1960 a spleen twice the normal size and containing a moderately increased number of lymphocytes was extirpated The splenectomy had hardly any effect on the need of transfusions In December 1960 2 discrete components were seen and with the new one (γG 1 3 g/100 ml) the total M component concentration was 2 3 g/100 ml Later the values noted for lymphocytes in the peripheral blood were the highest found (5 000—10 000/mm³) The patient deteriorated and died in April 1961 —Necropsy showed a picture compatible with lymphatic leukaemia though the changes in the lymph nodes as in the previously removed spleen were insignificant

Case 7 Female born in 1915 In 1960—61 she noticed swelling of several lymph nodes which was found on examination in May 1962 when the Hb was 13 4 g/100 ml and the W B C 8 100 of which 31 % were lymphocytes In July 1963 the hyperplasia of the lymph node had progressed and she was troubled by swelling in the region of pubis and perineum She was cachectic R B C 3 4 mill W B C 19 700 of which 82 % were lymphocytes in the bone marrow 85 % lymphocytes but no mast cell An M-component (γM 0 2 g/100 ml) was found in the serum and the urine contained 3 light-chain components X ray was given to the inguinal lymph nodes with good effect In February 1964 treatment with chlorambucil was started because of fatigue sweating loss of bodyweight increased lymph node hyperplasia enlargement of the spleen and a further increase of W B C (35 800 96 % lymphocytes) The drug had a good effect on the W B C which afterwards rarely exceeded 10 000 mm³ In the beginning her general condition also improved and the swelling of the lymph node regressed but only for a few months The concentration of the M-component increased from 0 4 to 1 9 from April to October 1964 but did afterwards not exceed 2 1 g/100 ml When she was admitted in January 1965 she had lost 16 kg since her last admission to hospital Her condition was afterwards characterised by cachexia increased tendency to infections anaemia and signs of hyperhaemolysis (reticulocytes 60 000—80 000/mm³ increased carboxyhaemoglobin) Finally hypercalcaemia (6 0 mEq/l) was noted as well as erosion of the compact bone of both the proximal humeral diaphyses She died in November 1965 —Necropsy Lymphatic leukaemia with lymphosarcoma—reticulum cell sarcoma and lymphocytic infiltration *inter alia* in the periosteum which was believed to explain the skeletal lesion

Case 9 Female born in 1886 She was admitted in 1953 because of fatigue loss of weight dizziness spontaneous haematoma and markedly enlarged spleen and liver R B C 2 4 mill W B C 358 000/mm³ of which 100 % were atypical cell varying in size and in form with scanty pale cytoplasm often structureless nucleus with one or several nucleoli in 18 % of the cells (Plate II 7) The number of thrombocytes was normal The bone marrow contained 90 % cells of the same type as those in the blood though the nuclear structure appeared more flocculant (Plate II 8) Because of suspected myeloblastic leukaemia busulphan was given with a good effect on her general condition enlargement of the liver and spleen and particularly on the blood picture (1954 R B C 3 8 mill W B C 16 000 of which 18 % were granulocytes) Serum electrophoresis from 1953 was not technically satisfactory but in 1954 2 components (γM) were noted and later a light chain component was found in the urine The concentration of the serum M component varied considerably and the highest value recorded was about 1 0 g/100 ml A fraction of the serum tended to gelate at room temperature In 1956 the patient s general condition became worse with fatigue loss of bodyweight slight enlargement of the spleen but no palpable liver and as previously no hyperplasia of the lymph nodes increasing W B C and gradually fever and death —The findings at necropsy were difficult to interpret Some fairly large lymphoid cells were seen in the spleen but no leukaemic infiltrates in the liver or spleen The only gross finding was a small amount of atypical tissue in and around one of the kidneys and ureters but no histological diagnosis was possible

Case 10 Male born in 1900 He was a steward and contracted syphilis in 1922 and in 1923 he had amoebic dysentery after which infection he occasionally had diarrhoea In 1943 achlorhydria was demonstrated when he sought advice because of diffuse abdominal pain In 1949

alpha₂ globulins The B_1 concentration in the serum was normal E S R 146 mm/1 hr and M component concentration 2 4 g/100 ml The sensitised sheep cell test was *negative* The patient was sent home in largely unchanged condition She was again admitted in 1960 Her *mobility* was markedly limited after a fracture of the femoral neck 7 months previously she had lost 5 kg in 2 years and was troubled by abdominal pain regurgitation and shortness of breath The lymph nodes and the spleen did not feel enlarged Haematologically the picture was unchanged since 1958 Bone marrow smears were of the same appearance and the M *component of the same concentration* Subfebrility disappeared in a ociation with antibiotic therapy but the N P N rose the patient deteriorated and died 3 months after admission —Necropsy Lymphocytic infiltration in the bone marrow in enlarged lymph nodes spleen normal sized liver kidneys fatty tissue and uterus signs of cardiac failure cholelithiasis and choledocholithiasis

Case XXII Female born in 1881 In 1953 she was admitted to hospital because of pneumonia She complained of swallowing difficulties which proved to be due to achalasia for which the patient refused operation From 1954 the patient consulted a practitioner mainly for her moderate shortness of breath and for checking the R B C which varied between 2 4 and 3 6 mill and which did not respond to iron and liver therapy The R B C had been checked already in *1950 and in July 1953 and* was then 4 1 respectively 4 0 mill When admitted because of rapidly progressing fatigue shortness of breath and *leg oedema in May 1965 she reported that* she had for 10 years been troubled by peripheral pain numbness and cyanosis when exposed to cold and that she had deteriorated during the last 6 months She was thin she had orthopnoea cyanosis and dyspnoea the heart rhythm was normal and the blood pre sure 160/80 there was pulmonary emphysema the liver was a hand breadth below the costal arch R B C 4 5—3 5 mill W B C normal as the number of thrombocytes in the bone marrow normoblastosis 17 % *lymphocytes* of which 17% had nucleoli 2 % plasma cells often in clusters and an increased number of mast cells The serum bilirubin was *intermittently increased (1 7* mg/100 ml) A pronounced tendency of the red blood cells to agglutinate in sampling microtubes prompted titration for cold agglutinins *(1 16 400)* and electrophoresis which showed an M component of 0 8 g/100 ml There were also signs of haemolysis in the form of decreased haptoglobin concentration and increased carboxyhaemoglobin After 2 weeks in hospital the patient died in pulmonary and cardiac in ufficiency — Necropsy Markedly dilatated oesophagus chronic *aspiration pneumonia* severe cardiac amyloidosis

Case XXII Male born in 1898 He had felt well until 1951 when he had fever for a month and pain and swelling of the knees and ankles and *when diabetes mellitus* was di covered In October 1957 he had for 2 weeks had fever cough and nose bleeding He was admitted to hospital in November and reported that the had lo t 7 kg in 8 months and that for 6 months he had been troubled by itching The spleen was palpated at the costal arch but otherwi e physical examination revealed nothing remarkable Hb 12 2 g/100 ml R B C 4 0 mill reticulocytes 2 4 % thrombocytes 82 000 and W B C 1 800/mm³ of which 98 % were atypical cells of which some were difficult to di tingui h from lymphocytes Similar cells (46 %) were seen in the bone marrow (Plate III *8 9*) where *also several large nucleolated nuclear fragments* 4 % plasma cells and numerous mast cells and normoblasts were found E S R 128 mm/1 hr and electrophoresis showed a serum M component of 0 7 g/100 ml Carboxyhaemoglobin was increased but Coomb s test was negative Skeletal x ray showed no lesions During chlorambucil therapy the number of granulocytes in the peripheral blood increased and the patient was sent home on this preparation in February 1958 Two weeks later fever supervened which was controlled by salicylic acid and he did not contact the doctor until after a further 2 weeks and then with purpura with a spleen extending *to 3 finger breadths below the costal arch* R B C 3 3 mill W B C 500 and thrombocytes falling from 80 000 to 26 000/mm³ The number of atypical *cells in the bone marrow had fallen* to 23 % the number of plasma cells was 0 2 % and as before numerous mast cells were found Treatment with ACTH and antibiotics had no effect on the serious course and the patient died one month later —Necropsy Wide pread and sometimes necrotic inflammation and monilia is in the pharynx and oesophagus punctate haemorrhages in the serous membranes *multiple* thrombi built up mainly of thrombocyte trabeculae and infarcts in the liver kidneys lungs thyroid musculature and skin increased haematopoiesis in the bone marrow liver pleen and lymph nodes diffuse increa e of pla ma cell *in the bone marrow*

CASE REPORTS CHAPTER V

Case 4 Male *born* in 1886 He had been admitted to hospital in 1954 becau e of abdominal discomfort Physical examination revealed nothing *abnormal* R B C 4 7 mill W B C 6 500/mm³ of which 40 % were lymphocytes E S R 4 mm/1 hr X ray howed colitis and osteoporosis *Becau e of the latter* finding sternal puncture was done and yielded a normal marrow

fatigue vomiting and diarrhoea On those occasions a successive increase was noted in the M component from 0 3 in 1954 to 0 8 g/100 ml in 1959 The bone marrow contained 31 % lymphocytes in 1955 45 % in 1957 and 47 % in 1959 In the last smear about one tenth of the lymphocytes were strikingly large and it also contained 5 % atypical cells (Plate III 1) which were found to the same extent in the peripheral blood but with darker blue cytoplasm and with oval or irregular nuclei with coarse and loose chromatin and sometimes with nucleoli (Plate III 2 3) In 1960 the patient fell acutely ill with abdominal pain and a state of shock and he was admitted to another hospital where explorative surgery was performed but the patient died one day later — Necropsy showed a spleen the size of a child's head a 3—4 cm thickened ventricular wall and enlarged lymph nodes along the aorta and in the liver hilus Histological examination of these organs gave a diagnosis of malignant lymphoma

Group I

Diagnosis or state of health at time of detection of M component	Diagnosis or state of health at last after examination or findings at necropsy
See case report	Fairly healthy
Thrombosis	Myelomatosis
Influenza	Myelomatosis ?
Diabetes mell	Diabetes mell
Cholecystitis	*Healthy*
Low back pain	Myelomatosis
Generalized pain	*Mental depr arthrosis*
Mental depr	Mental depr malignancy?
Recurr pneumonia	Healthy
Peptic ulcer sarcoidosis	Healthy (sarcoidosis)
Healthy blood donor	Healthy
Arthritis of undeterm etiol	Healthy
Pneumonia ozaena	Thrombosis
Mental depr arthrosis deform	Mental depr arthrosis *deform*
Cardiosel bronchitis	Cardiosel bronchitis
Peptic ulcer	Healthy
Pneumonia Paget's dis	Healthy (Paget's dis)
Frequency study hemiplegia	Healthy (*hemiplegia*)
Cerebrovascular lesion	*Healthy*
Rheum endocarditis cholecystitis	Rheum endocarditis
Routine control (mamm cancer)	Cystitis *arthrosis*
Rheum arthritis	Rheum arthritis pernic anaemia
Cerebral atrophy	Cerebral atrophy
Thyroid adenoma	Healthy
Spondylosis anaemia	Healthy (spondylosis anaemia)
Protracted broncho sinusitis	*Healthy*
Viral infect and or allerg react	Healthy
Diabetes mell	Healthy (*diabetes mell*)
Routine control (mamm cancer)	Metastasing cancer
Protracted pleuropneumonia	Healthy
Recurr broncho-sinusitis pneumonia	Healthy
Prostatic hypertrophy	*Cystitis*
Genetic study healthy	*Healthy*
Pharyngitis (anaemia hypothyr)	Healthy (anaemia hypothyr)
Health control	*Healthy*

cholecystectomy was done and in 19o2 he was *admitted because of fatigue nausea jaundice* and hepatomegaly The symptoms disappeared spontaneou ly and liver cirrhosis was suspected particularly because of a high consumption of alcohol In 1953 megaloblastic anaemia hista mine refractory achylia and a low serum vitamin B_{12} were found in association with an episode of diarrhoea accompanied by fatigue and lo s of bodyweight These symptoms di appeared on treatment with vitamin B_1 Roentgen examination of the stomach in 19o4 showed an atypical picture which at one of the later examinations was described as follows As at previous examinations the distal canal appears to be narrow and deformed On inflation this *part does not open in a normal way at the pyloric* junction As at previous examinations the mucosal relief in the region of the fundus is irregular A γM component was found in the serum in 1954 The patient was re admitted in 19o7 19o8 and 19o9 because of periods with

Table XIII

Case[1]	Sex *Age*	R B C *(mill)*	Bone marrow puncture Nr	*Plc* (%)	neutr (%)	Albumin (g/100 ml)	Gamma *fraction* (g/100 ml)	M component[3] *I* (g/100 ml)	*II*	*III*	Time[4] *(yr mth)*
Living											
1 556	F 82	3 0	4	21	8	1 9	0 5	4 1	2 6—2 5	γG	3—6
2 637	F 83	3 3	3	11	8	4 3	0 5	3 7	2 3—3 7	γG	5—11
3 392	F 63	3 8	3	16	10	3 7	0 3	3 0	1 2—3 0	γG	5—0
4 422	*F 60*	*4 6*	*5*	*19*	*5*	*4 0*	*0 3*	*2 8*	*2 5—2 6*	γG	*4—8*
5 94	F 67	4 2	6	9	5	4 4	0 6	2 7	1 5—2 6	γG	8—4
6 421	F 47	3 6	4	16	6	4 5	0 5	2 6	2 1—2 6	γA	4—9
7 141	F 65	3 7	4	3	4	5 1	0 4	2 5	2 5—2 2	γG	11—0
8 277	F 57	3 0	4	5	7	4 0	*0 3*	2 2	*1 5 1 9*	γG	*3—11*
9 718	M 66	4 0	3	2	6	3 5	1 0	2 0	1 7—1 6	γG	2—5
10 941	F 73	3 8	3	4	11	3 8	1 2	1 9	1 0—1 9	γG	1—2
11 28	M 48	4 6	4	6	6	5 1	0 7	1 7	1 0—1 7	γG	9—11
12 455	F 77	4 3	3	3	12	5 2	0 6	1 6	1 4—0 9	G	4—5
14 363	F 71	4 0	2	4	10	4 9	0 6	1 4	1 2—1 3	γG	4—4
15 491	F 63	4 0	3	4	4	4 6	0 8	1 4	1 1—1 1	γG	4—2
16 114	F 79	4 8	5	5	7	4 6	0 8	1 3	1 2—1 1	γG	10—4
17 619	F 63	4 8	3	4	8	4 3	0 7	1 3	1 3—1 2	γG	3—1
18 93	M 75	3 2	3	3	9	4 1	0 5	1 3	1 2—0 8	G	8—4
19 659	F 70	3 7	3	4	5	4 1	1 3	1 2	1 2—0 2	?	3—0
20 332	M 54	4 5	2	3	9	5 4	1 3	1 2	1 0—1 2	γG	4—5
21 884	F 64	4 9	3	3	*9*	*4 2*	*1 1*	*1 2*	*0 6—0 9*	γG	4—4
22 680	F 82	4 4	2	1	15	3 7	1 0	1 2	1 1 1 2	G	2—10
23 631	M 74	3 0	3	4	14	4 6	1 0	1 1	1 0—1 1	γG	3—1
24 963	M 62	4 5	3	3	16	4 9	0 6	1 1	1 1 1 0	γG	1 1
25 848	F 53	3 9	3	7	12	4 6	*0 8*	*1 0*	*0 9—1 0*	A	1—9
26 465	M 45	4 3	3	4	4	5 1	0 5	1 0	0 7—0 8	γG	4—3
27 558	M 61	4 3	1	1	6	4 3	1 3	1 0	1 0—0 0	?	3—9
28 *402*	M 65	3 0	0			2 4	1 6	1 0	0 5—0 0	γG	0—2
29 275	F 61	3 9	4	2	6	4 9	0 7	0 9	0 7—*0 5*	γA	*6—5*
30 209	F 57	4 2	3	2	5	4 5	1 0	0 9	0 8—0 8	γA	7—3
31 488	M 58	4 7	3	6	2	4 0	1 0	0 8	0 8—0 6	γG	4—0
32 951	*M 59*	4 3	4	3	17	5 3	0 8	0 8	0 8—0 8	γA	4—3
33 864	M 70	4 4	2	2	5	4 3	1 0	0 8	0 7—*0 8*	γG	1—9
34 608	F 62	4 4	3	2	9	5 0	0 6	0 8	0 8—0 6	γG	4—9
35 1068	F 64	3 7	3	1	6	4 2	0 7	0 8	0 2—0 8	γA	1—0
36 662	F 56	3 9	2	1	8	4 3	0 9	0 8	0 6—0 8	γG	2—10

fatigue, vomiting and diarrhoea. On those occasions a successive increase was noted in the M-component from 0.3 in 1954 to 0.8 g/100 ml in 1959. The bone marrow contained 31 % lymphocytes in 1955, 45 % in 1957 and 47 % in 1959. In the last smear about one tenth of the lymphocytes were strikingly large and it also contained 5 % atypical cells (Plate III 1) which were found to the same extent in the peripheral blood but with darker blue cytoplasm and with oval or irregular nuclei with coarse and loose chromatin and sometimes with nucleoli (Plate III 2, 3). In 1960 the patient fell acutely ill with abdominal pain and a state of chock and he was admitted to another hospital where explorative surgery was performed but the patient died one day later.—Necropsy showed a spleen the size of a child's head, a 3—4 cm thickened ventricular wall and enlarged lymph nodes along the aorta and in the liver hilus. Histological examination of these organs gave a diagnosis of malignant lymphoma.

Group I

Diagnosis or state of health at time of detection of M-component	Diagnosis or state of health at last after examination or findings at necropsy
See case report	Fairly healthy
Thrombosis	Myelomatosis
Influenza	Myelomatosis?
Diabetes mell.	Diabetes mell.
Cholecystitis	Healthy
Low back pain	Myelomatosis
Generalized pain	Mental depr. arthrosis
Mental depr.	Mental depr. malignancy?
Recurr. pneumonia	Healthy
Peptic ulcer. sarcoidosis	Healthy (sarcoidosis)
Healthy blood donor	Healthy
Arthritis of undeterm. etiol.	Healthy
Pneumonia. ozaena	Thrombosis
Mental depr. arthrosis deform.	Mental depr. arthrosis deform.
Cardioscl. bronchitis	Cardioscl. bronchitis
Peptic ulcer	Healthy
Pneumonia. Paget's dis.	Healthy (Paget's dis.)
Frequency study. hemiplegia	Healthy (hemiplegia)
Cerebrovascular lesion	Healthy
Rheum. endocarditis. cholecystitis	Rheum. endocarditis
Routine control (mamm. cancer)	Cystitis. arthrosis
Rheum. arthritis	Rheum. arthritis. pernic. anaemia
Cerebral atrophy	Cerebral atrophy
Thyroid adenoma	Healthy
Spondylosis. anaemia	Healthy (spondylosis. anaemia)
Protracted broncho-sinusitis	Healthy
Viral infect. and/or allerg. react.	Healthy
Diabetes mell.	Healthy (diabetes mell.)
Routine control (mamm. cancer)	Metastasing cancer
Protracted pleuropneumonia	Healthy
Recurr. broncho-sinusitis. pneumonia	Healthy
Prostatic hyperpl.	Cystitis
Genetic study. healthy	Healthy
Pharyngitis (anaemia. hypothyr.)	Healthy (anaemia. hypothyr.)
Health control	Healthy

Table XIII

Case[1]		Sex Age	R B C (mill)	Bone marrow puncture[2] Nr	Plc (%)	neutr (%)	Albumin (g/100 ml)	Gamma fraction (g/100 ml)	M component[3] I (g/100 ml)	II	III	Time[4] (yr mth)
37	731	F 68	4 4	2	1	14	4 1	0 6	0 8	0 8—0 5	γG	2—4
38	741	M 52	4 4	3	1	12	5 2	0 8	0 7	0 7—0 6	γG	7—9
39	511	F 25	4 2	2	1	23	4 8	0 6	0 7	0 6—0 5	γG	3—11
40	626	M 56	5 0	2	1	9	4 6	0 3	0 7	0 7—0 6	γG	3—1
41	669	M 48	3 7	4	16	15	5 0	1 4	0 6	0 6—0 4	γA	3—9
42	377	F 54	3 8	1	3	2	3 3	1 2	0 6	0 6—0 0	γG	0—1
43	801	F 50	3 9	3	2	6	4 8	0 6	0 6	0 6—0 4	γG	2—0
44	555	F 76	4 1	3	2	7	4 3	0 7	0 6	0 4—0 5	γG	3—6
45	917	M 66	4 0	2	2	10	4 2	1 4	0 6	0 5—0 6	γG	1—5
45a	504	M 58	4 1	1	1	10	2 9	3 5	0 6	0 6—0 0	?	0—8
46	869	M 43	4 4	3	1	15	4 6	0 6	0 6	0 3—0 5	γG	9—9
47	788	F 78	3 0	1	4	5	3 8	1 4	0 4	0 2—0 0	γG	0—11
48	781	F 65	2 2	3	3	10	4 4	0 9	0 4	0 4—0 2	γA	2—1
49	821	M 65	4 6	3	3	3	4 8	0 9	0 4	0 4—0 3	γG	1—10
50	787	M 59	4 2	3	3	11	4 9	1 5	0 4	0 2—0 4	γA	2—0
51	949	F 65	4 2	3	2	7	4 6	0 9	0 4	0 4—0 4	γG	1—2
52	650	M 75	4 4	3	2	14	4 3	0 6	0 4	0 3—0 3	γA	3—0
53	755	M 76	4 8	2	1	8	5 5	0 8	0 4	0 3—0 3	γG	2—3
54	509	F 44	4 1	2	1	8	5 0	0 9	0 4	0 2—0 3	γG	4—0
55	568	F 62	4 0	0			4 3	1 5	0 4	0 4—0 0	γG	2—8
56	81	M 63	4 5	5	4	9	4 5	0 8	0 3	0 2—0 3	γG	8—6
57	540	F 63	4 6	2	2	4	4 9	1 2	0 3	0 2—0 3	γG	6—2
58	591	F 65	4 2	3	2	12	4 7	0 8	0 3	0 2—0 2	γG	3—5
59	799	M 71	4 2	3	2	11	3 8	1 2	0 3	0 3—0 2	γA	2—0
60	994	F 76	4 0	3	2	5	5 3	1 1	0 3	0 3—0 3	γG	1—0
61	761	F 81	3 5	3	1	11	4 1	1 0	0 2	0 2—0 2	γA	2—3
62	779	F 50	3 7	0			4 4	0 5	0 2	0 2—0 0	γG	2—1
Dead												
63	278	F 63	2 6	3	4	10	1 7	0 6	3 9	2 5—3 9	γG	1—11
64	418	F 73	3 0	3	11	7	2 7	0 7	2 8	2 6—2 8	G	4—0
65	151	M 74	4 3	2	9	20	4 3	0 5	2 7	1 4—2 5	γG	7—5
66	118	M 68	3 9	2	5	8	4 5	0 4	2 2	2 0—2 0	γG	6—1
67	8	F 63	2 9	6	8	6	3 1	1 2	2 0	1 6—1 9	γG	8—6
68	1114	F 78	2 7	1	10	14	4 7	0 3	1 9		γG	0—1
69	459	F 84	3 0	2	6	8	4 0	0 3	1 7	1 7—1 5	γG	2—6
70	636	M 64	4 5	1	6	26	3 3	1 1	1 3		γG	0—1
71	395	M 78	4 4	1	<1	11	3 2	0 6	1 2	1 2—0 9	G	2—1
72	161	F 84	3 9	0			3 4	0 7	1 2		γG	0—8
73	529	M 62	4 2	0			3 1	0 8	1 1		γG	0—5
74	341	M 75	4 3	2	9	7	4 9	0 6	1 0	0 5—1 0	[illegible]	5—4
75	668	F 83	4 7	1	7		4 2	0 9	1 0	0 9—1 0	γG	2—0
76	165	F 75	4 5	1	6	10	2 8	0 5	1 0		γG	0—3
77	1218	F 71	3 3	1	4	11	2 1	1 0	1 0		γG	0—1
78	701	F 89	4 0	1	2	7	4 6	0 8	1 0	1 0—0 7	γG	1—4
79	295	M 84	3 7	1	<1	22	2 5	1 6	1 0	1 0—1 0	γG	0—5
80	440	M 55	3 0	3	10	8	2 5	0 9	0 9	0 9—0 0	G	1—2
81	875	M 66	3 7	1	3	4	3 6	0 4	0 9	0 9—0 8	γG	0—5
82	236	M 77	4 5	1	2	12	3 7	1 0	0 9		γG	0—3

(cont.)

Diagnosis or state of health at time of detection of M-component	Diagnosis or state of health at last after examination or findings at necropsy
Cholecystitis rheum. arthritis	Rheum. arthritis
Arthralgia	Healthy (arthralgia)
Non toxic goitre	Healthy
Pneumonia	Healthy
Encephalitis?	Encephalitis sequelae?
Glandular fever	Healthy
Uterine cancer	Healthy
Cholecystitis	Pancreatitis
Pleuritis	Healthy
See chapter VI	Fairly healthy
Healthy blood donor	Healthy
Fever of unknown origin	Senility
Pneumonia pernic. anaemia	Healthy
Prostatic hyperpl.	Healthy
Pneumonia	Healthy
Diaphragm. oesoph. hernia	Diaphragm. oesoph. hernia
Resp. infect. colon divert.	Prostatic hyperpl. colon divert.
Prostatic hyperpl.	Healthy
Glandular fever	Healthy
Encephalitis? rheum. arthritis	Rheum. arthritis
Arthralgia	Encephalomal. sequelae
Cardioscler. pulmon. sarcoidosis	Cardioscler. pulmon. sarcoidosis
Peptic ulcer	Cardioscler.
Osteomyelitis	Healthy
Pneumonia sinusitis	Healthy
Frequency study senile dementia	Senile dementia
Peritonitis (infect. endometriosis)	Healthy
Pneumonia gastric cancer	Gastric cancer
Thrombosis	Nephroscler. etc.
Cystopyelitis	Gastric cancer
Pneumonia	Rupt. aortic aneurysm cardioscler.
Arterioscler. etc.	Arterioscler. liver cirrh. etc.
Renal failure	Myeloma nephrosis?
Enteritis	Arterioscler.
Cardiac amyloid chron. pyelonephritis	Cardiac amyloid chron. *pyelonephritis*
Cardioscler. emphysema	Cardioscler. *pneumonia emphysema*
Cardioscler.	Cerebral haemorrh. emphysema
Urin. bladder cancer	Urin. bladder cancer
Peptic ulcer senility	*Bleeding peptic ulcer*
Frequency study senile dementia	Arterioscler. widespr. sarcoidosis
Senility	Pulm. emboly chron. pyelonephritis
Polyarteritis nod.	Polyarteritis nod. *myelomatosis*
Frequency study senility	Arterioscler. chron. cholecystitis
Pneumonia prost. cancer liver cirrh.	Prost. cancer liver cirrh.
Polyarteritis nod.	Polyarteritis nod.
Pulm. cancer	Pulm. cancer
Prost. cancer	Prost. cancer

Table XIII

Case[1]		Sex Age	R B C (mill)	Bone marrow puncture Nr	Plc (%)	neutr (%)	Albumin (g/100 ml)	Gamma fraction (g/100 ml)	M component[3] I (g/100 ml)	II	III	Time[4] (yr mth)
83	503	M 63	4 2	2	2	8	2 6	1 1	0 9	0 5—0 0	γG	3—1
84	655	M 87	3 6	1	1	18	5 0	0 8	0 9	0 8—0 8	γG	2—7
85	751	F 77	4 2	0			4 8	1 1	0 9		γG	0—2
86	797	M 82	3 9	2	3	13	3 6	0 3	0 8	0 8—0 7	γG	1—4
87	312	M 74	4 7	1	3	14	4 9	0 4	0 8	0 4—0 6	γA	5—10
88	477	M 73	4 4	1	1	30	4 9	0 5	0 8	0 8—0 7	γG	0—2
89	763	M 89	4 4	0			3 4	0 9	0 8		γG	0—2
90	653	M 89	4 3	1	1	26	4 9	0 9	0 7	0 7—0 7	γA	1—5
91	489	F 68	4 0	1	<1	5	4 9	0 5	0 7	0 5—0 7	γG	1—11
92	486	M 52	3 7	1	5	17	4 3	0 9	0 6	0 5—0 6	γA	0—8
93	607	M 80	3 7	3	3	16	4 6	0 6	0 6	0 6—0 6	γA	2—10
94	142	M 64	3 7	2	3	3	3 3	0 6	0 6	0 6—0 4	γG	4—10
95	360	F 77	4 4	1	2	22	5 0	1 1	0 6	0 4—0 6	γA	2—2
96	773	M 48	1 8	1	21	1	3 6	1 1	0 5	0 5—0 0	γG	0—1
97	478	M 47	3 9	2	7	8	1 1	0 3	0 5	0 4—0 5	γG	1—11
98	757	F 85	4 8	1	<1	16	3 9	1 3	0 5		γA	0—5
99	565	M 69	2 9	0			4 4	1 5	0 5	0 4—0 5	γG	1—0
100	1087	F 79	3 9	0			3 7	0 5	0 5		γG	0—5
101	843	M 86		0			3 7	0 8	0 5		γG	0—2
102	735	F 80	3 7	1	2	7	4 0	0 6	0 4		γA	0—3
103	48	M 62	4 8	2	<1	33	3 0	0 5	0 4	0 4—0 4	γG	2—5
104	688	M 90	4 1	1	1	19	4 5	1 0	0 3		γG	1—2
105	671	M 82	3 4	0			3 0	0 2	0 3		γA	0—8
106	E B	M 69	1 2	1	3	1	2 5	0 9	0 2		?	0—4
107	517	F 74	1 3	1	2	5	3 3	0 6	0 2	0 2—0 1	γG	1—6
108	638	F 78	3 6	0			5 0	0 6	0 2	0 2—0 0	γG	0—8

[1] Second column gives the immunoelectrophoretic number for identification

[2] Number of punctures performed plasma cells neutrophilic granulocytes at time of punctate with most plasma cells

[3] R B C albumin gamma fraction and M component (I) at the time of highest M component concentration recorded II first and last electrophore is III immunoglobulin class

[4] Interval between detection and last after examination first electrophoresis howing di appearance of the M component or death

(cont)

Diagnosis or tate of health at time of detection of M-component	Diagno is or state of health at la t after examination or findings at necropsy
Un_pec tongue ulcer	Liver cirrh
Frequency study healthy	Encephalomal cardiac amyloid
Frequency study senile dementia	Arterioscler liver cirrh et cancer
Arterio cler	Peri coll ren amyloid rupt aort aneury m
Myocard infarct	Cerebral haemorrh cardiac amyloid
Cholelithia i	Pulm embolies
Frequency study arterioscler deterior	Encephalomal
Frequency study emphy ema	Pulm cancer
Colonic diverticulosis mitr tenosis	Mitr steno is
Mitr steno is	Mitr teno is wide pr sarcoidosis
Arterio cler	Arterioscler prost cancer
Malabs colonic diverticulo is	Arterioscler chron pyelonephritis un pec enteritis low villi intest
Cardioscler	Enteritis gravis lipo arcoma
Tuberculo is	Tuberculo is
Peri coll amyloid	Cardioscler peri-coll amyloid
Ovarian cancer	Ovarian cancer
Ga tric cancer	Ga tric cancer
Senility ovarian kystaden	Arterioscler ovarian kystaden
Pancreatic cancer	Pancreatic cancer
Fracture senility Paget s dis bone	Arterio cler Paget s dis bone
Cerebr arterio cler	Arterio cler crural gangraena
Frequency study fairly healthy	Hepatic cancer
Frequency tudy *pro t cancer*	Pro t cancer
Pancytopenia ulc coliti p oriatic arthropathy *cardio cler*	Cardioscler ulc coliti myelomato i
Pancytopenia Paget s di bone	Chron pyelonephr Paget di bone
Melano arcoma	Melano arcoma

PLATE I





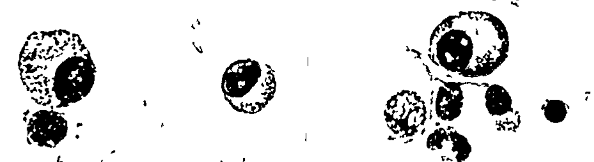




PLATE I

1 Ca e 64 (M-component γG 2 6 g/100 ml) Bone marrow containing 11 % pla ma cells Four pla ma cells with holes after crystalline inclu ions in the cytopla m and with nucleoli

2 3 Ca e 4 (M component γG 2 8 g/100 ml) Bone marrow containing 19 % pla ma cells Plasma cells with vacuolated cytopla m with crim on red pellets and large nucleoli

4 Case 85m (erum globulin 5 6 g/100 ml) Bone marrow containing 21 % plasma cell Four plasma cells of fairly normal appearance

5 6 Ca e 6 (M-component γA 2 6 g/100 ml) Bone marrow containing 16 % plasma cell Pla ma cells with pyknotic peripherally di placed nuclei Some cells with a fine reticular cytopla m some with partial compartment formation (6 upper left) and well developed compartments in one large cell (5)

7 Ca e 80 polyarteritis nodo a (M-component γG 0 9 g 100 ml) Bone marrow containing 10 % pla ma cells Three plasma cell howing polymorphi m and nucleoli The smear contained 22 % pla macellular reticulum cells of which the left cell i a fairly good example

8 Ca e 8 (M component γG 2 2 g 100 ml) Bone marrow containing 5 % plasma cells Three remarkably small pla ma cell

PLATE II

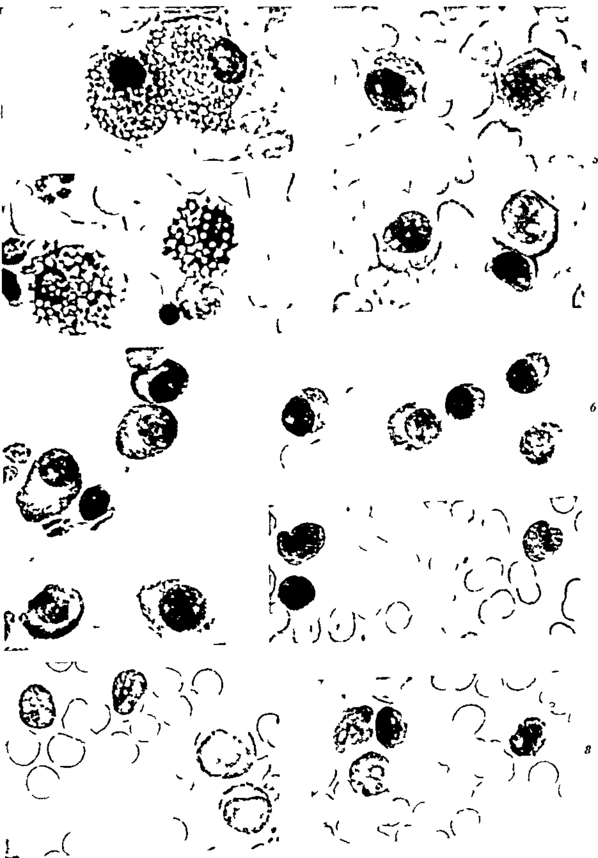


PLATE II

1 2 Case 41 (M-component γA 0 6 g/100 ml) Bone marrow containing 16 % plasma cells Mott cell with a grey material included in some vacuoles

3 6 Case 55m (M component γG 6 5 g/100 ml) Bone marrow containing 22 % plasma cells Plasma cells of largely normal appearance in a smear from a puncture performed late in the course

4 Bone marrow smear from a case of chronic lymphatic leukaemia (case 2 M component γM 0 4 g/100 ml) showing 2 lymphoblasts (right) and 2 lymphocytes of which at least the left one has a nucleolus

5 Case 3 (M-component γG 3 0 g/100 ml) Bone marrow containing 16 % plasma cells Four atypical plasma cells with a high nucleus/cell ratio centrally situated somewhat irregular nucleus with fine chromatin and some small nucleoli

7 8 A case of blast cell leukaemia (case 9 M component γM 1 0 g/100 ml) Atypical lymphoid cells in the peripheral blood (l) and in the bone marrow

PLATE III



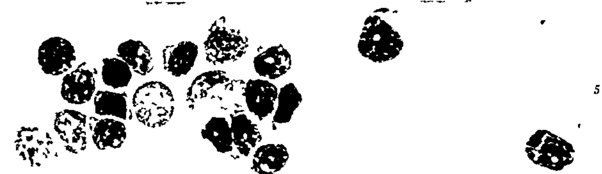






PLATE III

1—3 A case of lymphosarcoma (case 10, M-component γM 0.8 g/100 ml). Atypical cells in the bone marrow (1) and in the peripheral blood (2 cells to the right in 2 and in 3) with dark coarse chromatin and some with a nucleolus.

4 Various lymphoid reticulum cells in a case with a γM-component not presented in chapter IV.

5 Case XX (M-component γM 4.5 g/100 ml). Lymphoid reticulum cells in bone marrow smear containing 52 % lymphatic cells.

6, 7 Case XIV (M-component γM 6.7 g/100 ml). Atypical bone marrow cells, some resembling plasma cells, one with some large nucleoli (7), some with well demarcated intranuclear inclusions.

8, 9 Case XXIV (M-component γM 0.7 g/100 ml). Atypical bone marrow cells with irregularly shaped and sometimes lobulated nuclei with a fine chromatin and sometimes small nucleoli. The cytoplasm is pale, often with a fuzzy outline.

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GASTRIC MACROMOLECULES WITH SPECIAL REFERENCE TO INTRINSIC FACTOR

A Methodologic, Experimental and Clinical Investigation

BY

RAGNHILD GULLBERG

STOCKHOLM 1966

From the Department of Rheumatology (Head B Olhagen, M D),
Karolinska sjukhuset and King Gustaf V Research Institute
(Head G Birke M D), Stockholm, Sweden

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II Electrophoretic fractionation of B_{12} binders in gastric juice from patients with pernicious anemia and from controls Proc Soc exp Biol (N Y) *105* 62–66 1960

III A study of precipitating antibodies against human intrinsic factor Acta med scand *172* 385–388, 1962 In collaboration with S Kistner

IV Immunologic studies of intrinsic factor The reactions of experimentally produced antisera to human and hog intrinsic factor and of sera from pernicious anemia patients Acta med scand *174* 573–581, 1963 In collaboration with S Kistner

V Precipitating serum antibodies to intrinsic factor in pernicious anemia Acta med scand *180* 87–94, 1966 In collaboration with S Kistner L E Bottiger and U Ewaldsson

VI Separation and immunologic characterization of hog intrinsic factor in comparison with human intrinsic factor Acta med scand *180* 317–328 1966

These papers will be referred to under I–VI

INTRODUCTION

Human disease has often stimulated research work which has thrown light upon normal as well as pathologic functions. Investigations in pernicious anaemia for instance have led to the discovery of Castle's intrinsic factor in gastric juice the lack of which causes a defective absorption of "extrinsic factor". Vitamin B_{12} has become the commonly used term for extrinsic factor. It is produced solely by microorganisms. In man it is an essential food constituent. The intestinal absorption of B_{12} is limited to a few micrograms daily which approximates the daily requirement. Thus a defective intestinal absorption will easily cause a deficiency state which is characterized by megaloblastic anaemia, degenerative changes in the nervous system and other symptoms of severe cellular dysfunction. Therefore the detection of vitamin B_{12} and its therapeutic value was a great progress in medicine. Various forms of B_{12} or cobalamins and two coenzyme forms of the vitamin have been identified. Their labelling with radioactive cobalt has afforded an excellent tool for studies on the absorption and metabolic pathways of the vitamin. Under physiologic conditions vitamin B_{12} with a molecular weight of approximately 1500 cannot be absorbed from the intestine without previous binding to the intrinsic factor, a macromolecule secreted from the fundus mucosa of the stomach in man. This obligatory binding to a special transport substance has hitherto been found to be unique for the absorption of vitamin B_{12}. Little is known about the interaction of the intrinsic factor-B_{12}-complex with the intestinal mucosa cell. There is evidence that the intrinsic factor-B_{12}-complex is adsorbed to special receptors of the mucosa cells in the distal part of the small intestine by a physicochemical process requiring a pH above 5.7 and the presence of divalent cations. According to one hypothesis the absorption of the intrinsic factor-B_{12}-complex takes place by pinocytosis. The transport of B_{12} through the intestinal mucosa is a slow process of more than two hours. The fate of intrinsic factor after its attachment to the intestinal mucosa surface is virtually unknown. Enzymatic activity splitting the bond between B_{12} and intrinsic factor has been demonstrated in homogenates of intestinal mucosa but it is not restricted to the site of B_{12}-absorption and has been found in other organs as well. Thus the significance of this releasing factor in the absorption of B_{12} is uncertain. On the other hand there is no direct evidence that B_{12} is transported in complex with a carrier through the intestinal mucosa cell. It has been very difficult to identify, isolate and characterize intrinsic factor. Since its molecular structure is unknown it has not been possible to demonstrate whether fragments of the molecule are

absorbed from the gut Many patients treated orally with hog intrinsic factor produce specific antibodies to it This indicates that at least antigenic structures of the heterologous intrinsic factor are absorbed from the human intestine Furthermore, the immune response to hog intrinsic factor seems to be of significance to the state of refractoriness to orally administered hog intrinsic factor This implies a risk of relapse of B_{12} deficiency symptoms during hog intrinsic factor therapy and has led to withdrawal of such preparations from the pharmacopoeia in some countries However, no other noxious effects have been reported, and the use of hog intrinsic factor in the diagnosis and treatment of pernicious anaemia has added greatly to the knowledge of intrinsic factor The immunologic approach to the intrinsic factor problems seems promising It may be possible to determine the antigenic structure of intrinsic factor and subunits of the molecule The detection of autoantibodies to intrinsic factor and other cell constituents in the gastric mucosa suggests autoimmune mechanisms in the pathogenesis of gastric atrophy and pernicious anaemia This hypothesis is supported by observations of regeneration of gastric mucosa with return of secretory function during glucocorticoid therapy in patients with pernicious anaemia The vast literature on intrinsic factor was extensively reviewed in 1963 by Glass (15), with more than 1300 references The more recent literature has been reviewed by several authors (4 8 9 16 32 46 52, 57, 60)

Intrinsic factor is an example of a specific gastric macromolecule Several unspecific macromolecules, i e substances present also in other secretions or body fluids, have been demonstrated in gastric juice (vide 46) In the late 1950s the association between loss of serum proteins into the gastrointestinal lumen and the syndrome of 'hypercatabolic or idiopathic hypoproteinaemia became evident (vide 7 45, 46) Exudative or protein losing gastroenteropathies are now common terms These pathologic conditions besides studies on the albumin metabolism in burns (3), have incited investigations into the role of the gastrointestinal tract also in the normal catabolism of serum proteins (vide 7, 45 46)

The technique used in the collection of gastric juice is important to the results of qualitative and quantitative studies on gastric constituents In investigations of proteins in gastric juice a main problem is to prevent degradation by proteolytic enzymes, e g pepsin Contamination with saliva, duodenal contents or blood must also be avoided Furthermore, loss of gastric juice into the intestine during the collection period makes quantitation of gastric secretion difficult Solutions introduced into the stomach or administered drugs may influence not only the amounts of various constituents in the gastric juice secreted per unit of time but also the rate of gastric emptying

These aspects of the subject of gastric macromolecules are meant to form a general background of the present investigation the objects of which were

1 to work out a method for studying proteins in human gastric juice in an undegraded state (I)

2 to separate and characterize human gastric macromolecules in normal and pathologic conditions (I–IV VI)
3 to work out an electrophoretic and an immunologic method for in vitro assay of intrinsic factor in human gastric juice (II III)
4 to compare the therapeutically used hog intrinsic factor with human intrinsic factor (III IV VI) and
5 to investigate the occurrence and significance of precipitating antibodies to human and to hog intrinsic factor in patients with various diseases and medication (IV V VI)

1 Electrophoretic pattern of *proteins in human* gastric juice (I)

Acid hydrolysis and enzymatic digestion could have a profound influence on the proteins in gastric juice It is therefore necessary to study the gastric juice proteins in an undigested state and to ascertain to what extent differences found in *comparative studies of pathologic and* normal gastric juice are due to different stages of protein degradation

Methods and results

The digestion of gastric juice proteins by pepsin or other proteolytic enzymes active *in acid gastric juice was found* to be so rapid that it could not be prevented by immediate cooling and neutralization of the *continuously aspirated* gastric juice The acid gastric juice was therefore neutralized in vivo by intro*ducing sodium phosphate buffer* (pH 7 2 or 8 0 ionic strength 0 2) into the stomach *The admixture of saliva was pre*vented by a suction device *Before collec*tion of juice for analysis the stomach was rinsed with buffer or saline Each sample collected was checked to make sure that it *had a neutral or alkaline* pH Blood stained or bile stained juice was discarded The samples were immediately *cooled on ice* They were filtered through glass wool and centrifuged Concentration was performed by ultrafiltration *through collodion membrane*

The efficiency of intragastric neutralization in minimizing peptic digestion of the proteins was *demonstrated in paper* electropherograms of acid and non acid human gastric juice collected and treated *in different ways When sodium* borate buffer of pH 9 0 was used for electrophoresis using the technique described by *Glass et al* (11) *three* main protein fractions in normal gastric juice neutralized in situ, migrated to the anode The fastest migrating fraction was absent in gastric juice from patients with histamin fast achylia and in non acid gastric juice from subjects who had acid juice *after stimulation of the secretion* This component had the same electrophoretic mobility as crystalline bovine pepsin *This localization of pepsin in the electro*pherogram is in agreement with other studies (vide 46) A slowly migrating *fraction contained* the major portion of periodic acid-Schiff (PAS) positive substances indicating that it might re*present a mucoprotein complex* An intermediate fraction was found to correspond to serum albumin Albumin *could always be demonstrated in non* acid gastric juice and in acid juice neutralized *in situ*

The *electropherograms of normal* gastric juice neutralized in situ resembled the compressed pattern which *was earlier considered to be diagnostic* of gastric atrophy if obtained after stimulation of gastric secretion (11 12) By peptic *digestion in vivo or in vitro*

it was possible to produce a pattern similar to the one which was earlier re garded as normal (11 12) and which accordingly was characterized by break down products

Discussion

The results demonstrated the importance of intragastric neutralization in studies of macromolecules in gastric juice This has later been emphasized by several authors (6 22 26 28 38 39 46 51) The intragastric neutralization is essen tial e g for the identification of labelled proteins in acid gastric juice (vide 45 46) as has been shown in studies on the loss of serum albumin through the gastric mucosa in man using intrave nously injected ^{131}I albumin (59) By im munoelectrophoresis with its great re solution capacity several protein com ponents specific or unspecific to gastric juice have been demonstrated (23 26 28 51)

The paper electropherograms of mac romolecules stainable as proteins or carbohydrates of whole gastric juice did not seem to give information of practical importance in the differential diagnosis of various diseases Therefore in the further studies the main interest was directed to certain gastric macromole cules of current clinical interest i e albumin (59) and intrinsic factor (II–VI)

2 In vitro assay of human intrinsic factor by paper electrophoresis combined with autoradiography (II)

Grasbeck (18) in 1956 emphasized the influence of peptic digestion on intrin sic factor (IF) He attempted to inhibit the peptic activity in gastric juice by in vitro depepsination at pH 10 Two B_{12} binding components in acid human gastric juice were separated by starch column electrophoresis Only *one* B_{12}-binder was found in in vitro alkalinized juice It was shown to contain the IF active principle The other B_{12}-binder which migrated more rapidly to the anode was found to be augmented after *acid incubation* It was assumed to be a peptic autodigestion product of IF

Glass et al (13) in 1959 also demon strated two components binding *vitamin* B_{12} in acid human gastric juice by paper electrophoresis No attempts were made to inhibit peptic digestion *In* gastric juice from patients with *pernicious* anaemia they found only a rapidly migrating B_{12}-binder No comparison was made with gastric juice from patients with gastric achylia without clinical evidence of pernicious anaemia Glass et al suggested that either one or *both of the* B_{12}-binders represented or were associated with IF They *postulated* that IF might be a *complex of reactive* or prosthetic groups *on one or more* gastric mucoproteins *and their products* of degradation

In these and other earlier works (vide 15) *degradation* of the proteins in acid gastric juice was not efficiently pre *vented The* method of intragastric neu tralization was therefore used in the pres ent investigation of B_{12}-binding compo nents in human gastric juice

Methods and results

Radioactive cyanocobalamin was added in vitro to human gastric juice neutralized in situ. Paper electrophoresis was performed and the fractions which had bound the labelled B_{12} were demonstrated by autoradiography. Two B_{12} binding components, migrating to the anode *in sodium borate buffer of pH 9.0* were found in acid gastric juice but sometimes the fast migrating component could not be demonstrated. On the other hand, the slowly migrating B_{12} binder was always absent in gastric juice from patients with pernicious anaemia. In patients with gastric achylia and no clinical evidence of pernicious anaemia the two B_{12} binders were present after parasympathetic stimulation of gastric *secretion with carbamylcholine chloride* but sometimes the slowly migrating B_{12} binder was absent in the juice collected during fasting without stimulation. The finding of a slowly migrating B_{12} binder in gastric juice was in concordance with results of Schilling's urinary excretion test performed by a modified technique likewise using carbamylcholine chloride to stimulate the secretion of intrinsic factor (42).

Discussion

A distinctive electrophoretic pattern of B_{12} binding components in gastric juice was *obtained in pernicious anaemia patients* compared both with other patients *who had gastric achylia and with subjects* who had acid gastric juice. The results indicated the presence *of two B_{12} binding fractions* in human gastric juice, one related to IF and the other *unrelated to* IF. A non IF related B_{12} binder in gastric juice could explain previous unsuccessful attempts to use the B_{12} binding capacity of gastric juice in the diagnosis of pernicious anaemia (vide 15). It is suggested that the *electrophoretic method* could be used for in vitro assay of IF *in human gastric juice. Contrary to* previous in vitro methods it is not based *upon the effect of IF on the uptake of* B_{12} in intestinal mucosa (vide 15, 16). The electrophoretic method is easy to perform as a complement to other laboratory investigations on gastric juice. The assay of intrinsic factor would be of special interest in patients with gastric achylia, since acid gastric juice is very seldom devoid of IF (vide 15). The assay is a specific test for IF deficiency in contrast with the Schilling test, which is *clearly diagnostic of pernicious anaemia* only if it is demonstrated that a pathologic B_{12} absorption becomes *normal by* the addition of IF. It is well known that the Schilling test with or without human or hog IF could show pathologic values in various kinds of intestinal malabsorption syndromes, selective intestinal malabsorption of B_{12}, pancreatic achylia, infestation with botriocephalus latus, the refractory state to hog IF during medication with paraaminosalicylic acid, and in renal failure (vide 15, 24). Moreover, one advantage of using in vitro assay of IF in the diagnosis of pernicious anaemia is that radioactive isotopes are not given to the patient.

The *electrophoretic pattern* of B_{12}-binders in human gastric juice in normal *and pathologic conditions* has been confirmed by several authors using paper *electrophoretic and immunoelectrophoretic* techniques (14, 28, 46, 51, 52). The

two B_{12}-binders in gastric juice have also been identified in gastric mucosa (20 41) However the two B_{12}-binders in gastric juice were not separated from each other by starch-gel electrophoresis (50)

The pattern of B_{12}-binders in acid gastric juice that has not been neutralized in situ is more complex A third B_{12}-binder has been identified in non neutralized or in vitro neutralized acid gastric juice It has IF activity and seems to be immunologically identical with the native IF but behaves differently in electrophoretic and chromatographic experiments (14 15 46 51 52) This third B_{12} binder has not been found in gastric mucosa non acid gastric juice or in acid juice neutralized in situ (14 15 20 46 51 52) It has therefore been thought to be a degradation product of IF possibly a result of peptic digestion, but this could not be verified by in vitro experiments (52)

The terminology used by different authors for B_{12}-binding components in human gastric juice has recently been recorded by Glass (15 16) and Schultze & Heremans (46)

3 Identification of human intrinsic factor by the immunologic gel diffusion technique combined with autoradiography (III)

The antigenicity of IF was first demonstrated in 1958 by Taylor et al (58) They immunized rabbits with human and with hog IF preparations Antibodies were produced which were shown to inhibit the promoting effect of IF on the B_{12} absorption in pernicious-anaemia patients when they were given antiserum orally mixed with the IF preparation in a so-called in vivo inhibition test In experiments using the Ouchterlony technique several precipitin lines were obtained because impure IF was used

The identification of IF by the immunoprecipitin technique would enable demonstration of IF in mixtures of substances e g body fluids and tissue extracts Such techniques are also of value in the analysis of antigenic structure

Methods and results

Antiserum was produced by immunization of a rabbit with the IF related B_{12} binder of in situ neutralized human gastric juice after separation by electrophoresis in polyvinyl chloride using sodium borate buffer of pH 9 0 (5 36) For lack of a pure antigen for comparative immunologic studies, the reactions of B_{12} binding substances were selected for investigation by the autoradiographic technique When human gastric juice to which radioactive cyanocobalamin had been added reacted with the antiserum in gel diffusion experiments using the technique described by Ouchterlony (37) many precipitin lines were obtained but only one of them contained radioactive B_{12} At immunoelectrophoresis (17) the precipitate containing the labelled B_{12} was shown to correspond to the slowly migrating B_{12}-binder of human gastric juice the one which is absent in pernicious anaemia (II) and which has been shown by Gräsbeck (18) to be a carrier of IF activity No B_{12}

Methods and results

Radioactive cyanocobalamin was added in vitro to human gastric juice neutralized in situ. Paper electrophoresis was performed and the fractions which had bound the labelled B_{12} were demonstrated by autoradiography. Two B_{12} binding components, migrating to the anode in sodium borate buffer of pH 9.0 were found in acid gastric juice, but sometimes the fast migrating component could not be demonstrated. On the other hand the slowly migrating B_{12} binder was always absent in gastric juice from patients with pernicious anaemia. In patients with gastric achylia and no clinical evidence of pernicious anaemia the two B_{12} binders were present after parasympathetic stimulation of gastric secretion with carbamylcholine chloride, but sometimes the slowly migrating B_{12} binder was absent in the juice collected during fasting without stimulation. The finding of a slowly migrating B_{12} binder in gastric juice was in concordance with results of Schilling's urinary excretion test performed by a modified technique, likewise using carbamylcholine chloride to stimulate the secretion of intrinsic factor (42).

Discussion

A distinctive electrophoretic pattern of B_{12} binding components in gastric juice was obtained in pernicious anaemia patients compared both with other patients who had gastric achylia and with subjects who had acid gastric juice. The results indicated the presence of two B_{12} binding fractions in human gastric juice, one related to IF and the other unrelated to IF. A non IF related B_{12} binder in gastric juice could explain previous unsuccessful attempts to use the B_{12} binding capacity of gastric juice in the diagnosis of pernicious anaemia (vide 15). It is suggested that the electrophoretic method could be used for in vitro assay of IF in human gastric juice. Contrary to previous in vitro methods it is not based upon the effect of IF on the uptake of B_{12} in intestinal mucosa (vide 15, 16). The electrophoretic method is easy to perform as a complement to other laboratory investigations on gastric juice. The assay of intrinsic factor would be of special interest in patients with gastric achylia, since acid gastric juice is very seldom devoid of IF (vide 15). The assay is a specific test for IF-deficiency, in contrast with the Schilling test, which is clearly diagnostic of pernicious anaemia only if it is demonstrated that a pathologic B_{12} absorption becomes normal by the addition of IF. It is well known that the Schilling test with or without human or hog IF could show pathologic values in various kinds of intestinal malabsorption syndromes, selective intestinal malabsorption of B_{12}, pancreatic achylia, infestation with botriocephalus latus, the refractory state to hog IF, during medication with paraaminosalicylic acid, and in renal failure (vide 15, 24). Moreover, one advantage of using in vitro assay of IF in the diagnosis of pernicious anaemia is that radioactive isotopes are not given to the patient.

The electrophoretic pattern of B_{12} binders in human gastric juice in normal and pathologic conditions has been confirmed by several authors using paper electrophoretic and immunoelectrophoretic techniques (14, 28, 46, 51, 52). The

strated by precipitin cross reactions with antibodies in rabbit antisera to hog and human IF preparations respectively

The terms organ specific and species-specific will in the following be used in their immunologic sense indicating similarities and differences of antigens in organs from different species viz of IF from man and hog and of antibodies directed to or cross reacting with these antigens

5 Precipitin reactions of human and hog intrinsic factor with antibodies in patients' sera (IV, V, VI)

Many pernicious anaemia patients treated with hog IF orally have been found to become refractory to orally administered IF from the hog or the wild boar but not to IF from man or rat (47 48 50) This refractoriness could be more rapidly produced by hog IF preparations with a high content of B_{12} than by the same preparation with a low B_{12}-content indicating that it was the IF in complex with B_{12} which was absorbed from the intestine and induced the antibody production (50) Variations in the refractory state during withdrawal and recommencement of the hog IF therapy were interpreted as signs of an immunologic anamnestic response (50) The view that the acquired resistance to hog IF was due to an immune mechanism was supported by the observations that patients sera reacted with hog IF in in vivo inhibition and haemagglutination tests (35 48 50) But this could not be confirmed by using other immunologic in vitro techniques (33) Furthermore pernicious anaemia patients never treated with hog IF were found to have serum antibodies that suppressed the effect of both human and hog IF in the in vivo inhibition tests (49 55) These antibodies did not cause refractoriness either to human or to hog IF Serum antibodies reacting with human IF have also been demonstrated by various in vitro techniques (vide 15 16) In 1962 antibodies reacting with an IF B_{12}-complex were demonstrated in 20 to 30 per cent of patients with pernicious anaemia by Jeffries et al (31) and Taylor et al (56) using electrophoretic retention techniques In their work Jeffries et al also presented an autoradiogram of an Ouchterlony experiment showing the precipitin reactions of a B_{12} binding component in human gastric juice with antibodies in sera from two patients with pernicious anaemia but they did not report the incidence of positive precipitin reactions in their patients By other techniques based on the observation that the B_{12} binding of IF is inhibited by antibodies to IF autoantibodies to IF have been demonstrated in 30 to 60 per cent of patients with pernicious anaemia (1 2) In the B_{12}-binding inhibition test as in the in vivo inhibition the autoantibodies cross-reacted with hog IF (vide 29)

Methods and results

The sensitivity of the immunoprecipitin methods was increased by adding radio-

binder of the same antigenic structure could be demonstrated in human serum, bile or liver (IV)

The results indicated that human intrinsic factor could be identified by immunoprecipitin techniques combined with autoradiography Consistent results have been published by Hurlimann (28) and Simons et al (51)

4 Comparison of the antigenic structure of human and hog intrinsic factor (III, IV, VI)

The concept of species specificity and non specificity of IF was first used for the IF dependent mechanism of intestinal B_{12} absorption Guinea pig IF for example, is active in the guinea pig alone, whereas IF from several other species is active in the guinea pig On the other hand, rat IF is active in man, monkey, hamster and guinea pig Therefore, the guinea pig has been called the universal IF acceptor and the rat the 'universal IF donor These findings suggest that the species specificity of the IF function depends upon a similarity not only in the structure of IF but also in a special intestinal absorption mechanism (vide 15)

Hog IF is active in man, which accordingly indicates that hog IF resembles human IF in its molecular structure and fits with the IF mediated absorption mechanism of B_{12} in the human intestine Hog IF has therefore been used for replacement therapy in pernicious anaemia (vide 15)

Taylor et al (53) found that rabbit antiserum to hog IF diminished the effect not only of hog but also of human IF in the in vivo inhibition test in a patient with pernicious anaemia This was further evidence of a similarity in the structure of IF from the two species

In simple precipitin tests and in Ouchterlony experiments a protein component in a hog IF preparation cross reacted with antibodies in rabbit anti serum to a human IF preparation (53 54) However, since impure IF without labelled vitamin B_{12} was used it could not be concluded that the precipitin cross reactions actually involved IF or even a B_{12} binding substance

Methods and results

In the present work, using a microprocedure (58) of the Ouchterlony technique (37) and immunoelectrophoresis (17) combined with autoradiography it was shown that a B_{12} binder in human and in hog IF preparations cross reacted with rabbit antisera to hog and to human IF preparations respectively

These B_{12} binders could be identified as IF related by immunologic comparative studies, using purified human IF e g Grasbeck s IF active B_{12} binder S (19) which has been demonstrated to fulfil several criteria of purity (21), and a purified IF active hog B_{12} binder from which B_{12} binders unrelated to IF had been removed by gel filtration

Discussion

The similarity in antigenic structure of human and hog IF was clearly demon

in hog liver homogenate and bile (IV) Therefore attempts were made to separate these two B_{12}-binders so as to enable further characterization and determination of their relationship to IF

Methods and results

The two components which were binding the bulk of added radioactive cyanocobalamin in the hog IF preparation WES 942 (Lederle laboratories Pearl River N Y) were separated by recycling gel filtration on Sephadex G 200 using the technique described by Flodin (10) Killander (34) and Porath et al (40) By comparison with reference substances it was shown that the larger component called A would have a molecular size between that of human gamma G globulin and that of albumin The smaller component B would have a molecular size similar to that of human albumin and slightly larger than that of human IF in gastric juice neutralized in situ In paper electrophoresis using sodium borate buffer of pH 9 0 component B moved more slowly than A to the anode whereas it moved slightly faster than A in barbiturate buffer of pH 8 6 Component B alone possessed IF activity in the Schilling test in patients with pernicious anaemia Component B was also the only one which cross-reacted with antibodies in rabbit antisera to human IF preparations and which reacted with antibodies in sera from patients treated with hog IF orally In reactions with rabbit antisera to the hog IF preparation WES 942 the precipitin line of component B could be found to fuse partially into a line caused by a fraction in component A A similarity in antigenic structure of the separated component A to a B_{12}-binder in hog bile was demonstrated by precipitin reactions of identity, using rabbit antisera to the hog IF preparation WES 942 and to hog bile

Discussion

Properties specific for IF were found in only a minor fraction of the B_{12} binding substances in the hog IF preparation WES 942 This fraction was separated by recycling gel filtration on Sephadex G 200 and designated as component B It seemed immunologically homogeneous with respect to B_{12}-binding substances whereas signs of heterogeneity were observed in the elution fractions containing the B_{12}-binding component A Aggregation products of component B might have elution properties similar to those of component A in gel filtration There is evidence that IF B_{12}-complexes have a tendency to form dimers (21 25)

Highly potent hog IF has earlier been prepared by using several purification steps (vide 15 16) but these preparations were not characterized immunologically The published reports on the physicochemical properties of IF are discrepant (vide 15 16) mainly because of difficulties in preparing IF in a pure and well-defined form The present results could agree with those of Holdsworth (27) who separated one IF active and one inactive B_{12}-binder from a hog pyloric mucosa preparation Irvine (29) in 1966 demonstrated that the B_{12}-binding of seven different hog IF preparations including WES 942 was inhibited by autoantibodies to IF in patients sera but only a minor part of the B_{12}-binding substances in the prepa

active cyanocobalamin of high specific activity to the antigens In about 60 per cent of 31 patients with pernicious anaemia treated with hog IF orally, a precipitin reaction with a B_{12}binder in hog IF preparations was demonstrated Hog IF did not normalize the Schilling test in pernicious anaemia patients, who had antibodies to the hog antigen In patients without pernicious anaemia, who had received hog IF orally, a positive reaction to hog IF was also demonstrated more often than in controls without known hog IF intake In about 10 per cent of 78 pernicious anaemia patients but in none of the 187 control subjects antibodies to a B_{12} binder in human IF preparations were shown Reactions both to human and to hog IF seldom occurred

Discussion

Precipitating serum antibodies to a human IF B_{12} complex were found only in pernicious anaemia The precense of antibodies to a hog IF B_{12} complex was related to oral hog IF treatment and to refractoriness to oral hog IF The results support the view that the acquired resistance to orally administered hog IF might have an immunologic basis They also agree with the hypothesis formed by Schwartz (50) that the genesis of patients antibodies to human and to hog IF is different The antibodies reacting with human IF are supposed to be autoantibodies The antibodies reacting with hog IF appear to be directed to heterologous IF absorbed from the intestine

Besides species specific antigenic sites and possible hidden antigenic determinants which could be exposed by splitting of the molecule there is evidence of an important difference in the antigenic structure of human and of hog IF which induce antibody production in man In the human IF, which might induce antibody production if released from the gastric mucosa undergoing cellular destruction, the B_{12} binding site is free On the other hand it is hidden by bound B_{12} in the hog IF B_{12} complex which is assumed to be absorbed from the gut This would influence the results of different immunologic methods, depending on the use of the IF or of the IF B_{12} complex as antigen (1 43) It could be one explanation of the low frequency of precipitating antibodies reacting with human IF B_{12}-complex in pernicious anaemia sera and of the species specificity in these reactions compared with the results of methods which are based on the blocking of B_{12} binding by IF antibodies

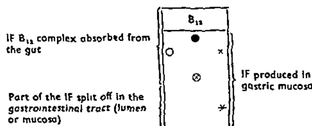
6 Separation by gel filtration and immunologic characterization of hog intrinsic factor in comparison with human intrinsic factor (VI)

It was shown that only a minor B_{12} binding fraction in commercial hog IF preparations reacted with antibodies in sera from hog IF treated patients and cross reacted with antibodies in rabbit antisera to human IF preparations (IV) The major B_{12} binding fraction showed immunologic similarity to a B_{12} binder

7 Hypothesis on the antigenic sites of the human and the hog intrinsic factor inducing antibody production in man (VI)

The immunologic reactions of human and hog IF and IF B_{12}-complexes with antibodies in patients sera compared with those in experimentally produced rabbit antisera and the effects of IF antibodies on the IF mediated B_{12} absorption (Table I) might suggest possible antigenic sites of IF inducing the antibody production (Fig 1)

Autoantibodies to IF have frequently been demonstrated in patients with pernicious anaemia (Table I) by techniques based on the blocking of B_{12}-binding to IF by antibodies to IF Autoantibodies reacting with the IF B_{12}-complex used as antigen in the electrophoretic retention test and in immunoprecipitin reactions have been less often demonstrated (Table I) These observations have suggested that the autoantibodies to IF are directed mainly to the B_{12}-binding site of the molecule (r 43) or its immediate surroundings The organ specificity of the B_{12} binding site of IF is evidenced by the observations that autoantibodies to human IF cross react in B_{12}-binding inhibition tests with IF from other species and do not react with B_{12}-binders



○ Organ specific antigenic site of IF

● Organ specific antigenic site of the B_{12} binding site of IF

× Species-specific antigenic site of IF absorbed from the gut in complex with B_{12} in man

✳ Species specific antigenic site of IF produced in gastric mucosa

○ Antigenic site of the IF B_{12} complex absorbed from the gut hidden in the IF produced in gastric mucosa may be organ or species-specific

○ ⊗ Immunologic tolerance to these antigenic sites of the autologous IF absorbed from the gut in complex with B_{12}

● ✳ Antigenic sites of the autologous IF which might induce autoantibody production if IF is released from a gastric mucosa undergoing cellular destruction

× (species-specific) and ○ Antigenic sites of the absorbed hog (heterologous) IF B_{12} complex which might induce antibody production in man

● Antigenic site of hog IF which might cross react with patients autoantibodies to IF

○ Antigenic sites of human and hog IF B_{12}-complexes which might cross react with rabbit antisera to hog and human IF respectively

Fig 1 Hypothesis of antigenic sites of human and hog intrinsic factor (IF)

rations studied cross reacted with these antibodies The identification of IF and other substances in IF preparations by the immunoprecipitin technique could serve as a guide in purification experiments *In studies on IF it is often important* to make sure that the preparations used do not contain B_{12}-binders other than IF In the interpretation of earlier results the possibility must be considered that B_{12} binding components unrelated to IF might have led to false conclusions as regards IF

The recycling gel filtration on Sephadex G 200 with continuous registration of radioactivity in the eluate is well suited for the purification of IF in complex with radioactive vitamin B_{12} The separation of IF not in complex with B_{12} *would* be possible under well standardized conditions The guiding of the recycling could be facilitated by the use of an indicator substance with elution properties similar to those of IF, when the amount of IF applied to the column is too small to be measured as protein in the eluate by conventional methods

TABLE I *Intrinsic factor antibodies*

	Relationship to: pernicious anaemia (p a)	Relationship to: oral hog IF intake	Relationship to: retractoriness to oral human IF	Relationship to: retractoriness to oral hog IF	In vivo inhibition test: reaction with homologous IF	In vivo inhibition test: cross reaction with heterologous IF (human, hog)	Immunologic in vitro tests: reaction with homologous IF	Immunologic in vitro tests: reaction with homologous IF B_{12} compl	Immunologic in vitro tests: cross reaction with heterologous IF (human hog)	Immunologic in vitro tests: cross reaction with heterologous IF B_{12}-compl (human, hog)
Autoantibodies to IF in man[a]	+	−	−	−	+	+	+ 30–60% in p a	+ 10–30% in p a	+	−
Antibodies to ingested hog IF in man[b]	−	+	−	+				+		−
Antibodies to injected hog IF in man[c]				−	+					
Antibodies to injected human IF or IF B_{12} complex in rabbit[d]					+			+		+
Antibodies to injected hog IF or IF B_{12} complex in rabbit[e]					−	+		+		+

[a] Taylor 1959 (55) Schwartz 1960, 1962 (49, 50) Jeffries et al 1962 (31) *Taylor et al* 1962 (56) Roitt et al 1964 (43) Abels et al 1963 (1) Ardeman et al 1963 (2) Gullberg et al 1963 1966 (IV V) Samloff et al 1965 (44) Irvine 1966 (29)

[b] Schwartz et al 1957 (47) Schwartz 1958 1960 1962 (48 49 50), Lowenstein et al 1961 (35) Gullberg et al 1963 1966 (IV, V), Gullberg 1966 (VI)

[c] Kaplan et al 1963 (33)

[d] Taylor et al 1958 (53) *Gullberg et al* 1962, 1963 (III IV) Simons et al 1963 (51) Hurlman 1963 (28) Simons 1964 (52)

[e] Taylor et al 1958 (53) Lowenstein et al 1961 (35) Gullberg et al 1963 (IV) Gullberg 1966 (VI)

7 Hypothesis on the antigenic sites of the human and the hog intrinsic factor inducing antibody production in man (VI)

The immunologic reactions of human and hog IF and IF B_{12}-complexes with antibodies in patients sera compared with those in experimentally produced rabbit antisera and the effects of IF antibodies on the IF mediated B_{12} absorption (Table I) might suggest possible antigenic sites of IF inducing the antibody production (Fig 1)

Autoantibodies to IF have frequently been demonstrated in patients with pernicious anaemia (Table I) by techniques based on the blocking of B_{12}-binding to IF by antibodies to IF Autoantibodies reacting with the IF B_{12} complex used as antigen in the electrophoretic retention test and in immunoprecipitin reactions have been less often demonstrated (Table I) These observations have suggested that the autoantibodies to IF are directed mainly to the B_{12}-binding site of the molecule (1 43) or its immediate surroundings The organ specificity of the B_{12} binding site of IF is evidenced by the observations that autoantibodies to human IF cross react in B_{12} binding inhibition tests with IF from other species and do not react with B_{12}-binders

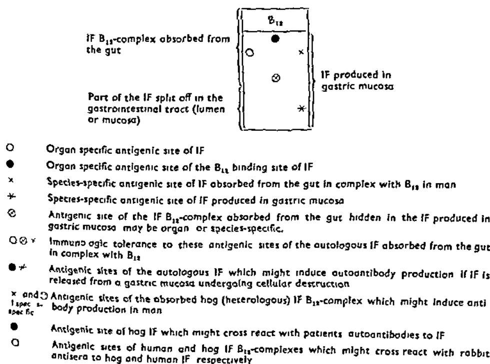


Fig 1 Hypothesis of antigenic sites of human and hog intrinsic factor (IF)

other than IF (1, 29) The B_{12} binding site of IF could not be the only organ specific antigenic structure of IF, as *cross reaction occurs when human or hog* IF in complex with B_{12} is tested against experimentally produced rabbit antisera to IF (Table I, Fig 1) By contrast, the autoantibodies to human IF were found *not to cross react with the hog IF in complex* with B_{12} (Table I) The patients' antibodies to ingested hog IF have also been found to be species specific in immunologic in vitro studies, using an IF B $_{12}$ *complex as antigen (Table I)*

There are reasons to believe that the hog IF antibodies in the patients are directed to a form of hog IF absorbed from the gut in complex with B_{12} (50) The *antigenic determinants of the* B_{12} binding site would then be hidden by bound B_{12} Accordingly, the antibodies to ingested hog IF could be expected to be species specific even in reactions with IF not in complex with B_{12} The question remains why organ specific sites of *the hog IF* B_{12} complex *do not induce* the production of antibodies which would cross react with other organ specific determinants of human IF It may be assumed that the hog IF, being active in man, is metabolized by largely the same process as the human IF in the gastrointestinal tract of man If this were true the antibody production to ingested and absorbed hog IF in complex with B_{12} would be indirect evidence for the absorption of structures of the human *IF* B_{12}*-complex from the intestine* in man An immunologic tolerance to such an absorbed part of the *autologous IF* B_{12} complex could be presumed The antibodies to the hog IF B_{12} complex absorbed from the gut would then be formed only *against species specific anti*genic sites of the heterologous IF B_{12}-complex (Fig 1) and cross reaction with human IF would not occur, provided that the immunologic tolerance has not been broken This would *agree with the species specificity in the refractory state to orally* administered *hog IF (Table I)*

Postulating an immunologic tolerance only to antigenic sites of a part of the autologous IF absorbed from the gut in complex with B_{12}, autoantibodies could *be produced to other antigenic sites of* the IF released from the gastric mucosa undergoing cellular destruction (Fig 1) As mentioned in the foregoing there is evidence that the B_{12} binding site of IF *is of importance in inducing autoanti*body production (Table I, Fig 1) There may also be other antigenic differences between the form of IF produced in the gastric mucosa and the one absorbed from the intestine (Fig 1) which could explain reactions of autoantibodies with *human IF in complex with* B_{12} *(Table I)* These theories could agree with the findings that the autoantibodies to IF in pernicious anaemia patients cross react in vitro with hog IF but not with hog IF in complex with B_{12} (Table I) that human and hog IF in complex with B_{12} *promote the intestinal absorption* of the bound B_{12} also in patients with autoantibodies to IF *(Table I) and that the* promoting effect of IF on the B_{12} absorption is inhibited by autoantibodies to IF when they are mixed in vitro with the human or the hog IF and given orally *together* with the B_{12} to a pernicious anaemia patient in the in vivo inhibition test *(Table I) Analogous effects on the*

IF mediated absorption of B_{12} have been observed in a study of antibodies produced in a pernicious anaemia patient by parenteral administration of hog IF without B_{12} (Table 1)

The theory of immunologic tolerance to antigenic sites of a part of the autologous IF absorbed from the intestine in complex with vitamin B_{12} is based upon few known facts Several of the assumptions made relate to problems that are subjects of controversy Therefore the postulations on the antigenic sites of human and of hog IF inducing antibody production in man should merely be regarded as details of a working hypothesis

SUMMARY

1. The method of in situ neutralization of gastric juice was introduced as a means of minimizing degradation of the macromolecules in acid gastric juice. Acid and non-acid human gastric juice collected and treated in different ways was studied by paper-electrophoresis. Albumin was found to be one of the main protein components in acid gastric juice neutralized in situ, as well as in non-acid juice (I).

2. By paper electrophoresis combined with autoradiography two vitamin B_{12} binding components were demonstrated in acid human gastric juice neutralized in situ, and in the juice obtained after parasympathetic stimulation of the secretion in patients with gastric achylia and no clinical evidence of pernicious anaemia. The slowly anodically migrating B_{12} binder was invariably absent in gastric juice from patients with pernicious anaemia, but the fast migrating B_{12} binder was found. The results indicated the presence of one B_{12} binder related to intrinsic factor (IF) and one B_{12} binder unrelated to IF in the normal gastric secretion. This implies a new principle for in vitro assay of IF in human gastric juice. The electrophoretic method was found to be suitable as a diagnostic test in pernicious anaemia (II).

3. IF in human gastric juice was immunologically identified by the Ouchterlony technique and immunoelectrophoresis combined with autoradiography using rabbit antiserum to electrophoretically purified IF from human gastric juice (III).

4. Precipitin cross reactions of human and hog IF B_{12} complexes with antibodies in experimentally produced rabbit antisera to hog and human IF preparations were demonstrated, indicating a similarity in the antigenic structure of the IF from the two species (III, IV, VI).

5. The occurrence of precipitating antibodies reacting with B_{12} binding components in complex with radioactive vitamin B_{12} was studied in patients with various diseases and medication. Antibodies reacting with the IF related B_{12} binder in human gastric juice were found only in patients with pernicious anaemia. Antibodies reacting with a B_{12}-binder in hog IF preparation were found also in patients without clinical evidence of pernicious anaemia. A relationship between hog IF intake and antibodies reacting with the hog antigen was demonstrated. Hog intrinsic factor did not normalize the Schilling tests in pernicious anaemia patients who had antibodies to the hog antigen. The patients' sera seldom reacted both with the human and with the hog antigen, indicating a species specificity of the patients' antibodies in their reactions with IF B_{12} complexes. The results supported the theory of a different genesis of the antibodies to human and to hog IF in per

nicious anaemia The antibodies reacting with human IF were supposed to be autoantibodies The antibodies reacting with a B_{12}-binder in hog IF preparation appeared to be directed to heterologous IF absorbed from the intestine (IV V, VI)

6 Using the hog IF concentrate WES 942 (Lederle) as starting material two B_{12} binding components were separated by recycling gel filtration on Sephadex G 200 The components were characterized by gel filtration and by electrophoresis and compared with reference substances Only the minor component possessed IF activity in Schilling tests in patients with pernicious anaemia and was shown to be antigenically similar to human intrinsic factor It was also the only component that reacted with antibodies in serum from patients treated with hog IF orally The major component which had a larger molecular size showed antigenic similarity to a B_{12}-binder in hog bile. (VI)

7 The immunologic results are discussed with reference to a theory that structures of IF in complex with B_{12} are absorbed from the intestine It is assumed that man is immunologically tolerant to an absorbed form of the autologous IF in complex with vitamin B_{12} A hypothesis of antigenic sites of human and of hog IF inducing antibody production in man is presented (VI)

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SMALLPOX OUTBREAK AND VACCINATION PROBLEMS IN STOCKHOLM, SWEDEN 1963

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CHAPTER I

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in collaboration — in the epidemiological field work and control measures in the hospitals —, with

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[1] National Bacteriological Laboratory
[2] Central Bacteriological Laboratory of Stockholm City
[3] Southern Hospital Stockholm
[4] Stockholm Board of Public Health
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[6] Stockholm Hospital for Infectious Diseases
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[8] Stockholm City Medical Services Administration
[9] Danderyd Clinic for Infectious Diseases, Stockholm County
[10] Stockholm County Administration Board

Introduction A historical survey

The first wide scale epidemic of small pox in Sweden that has been reported occurred in 1736 (1) The disease later spread throughout the country It was estimated that 270,000 persons died of smallpox in Sweden between 1749 and 1800, i e more than 10 % of the population

In the middle of the 18th century attempts to prevent the spread of small pox were made by means of quarantine, isolation and disinfection These measures, however, had no decisive effect Smallpox epidemics were common in Europe during that century

When it was realized that an attack of smallpox gave protection against new attacks of the disease, smallpox houses were established in Sweden in the 1750 s In these children were deliberately exposed to the apparently inevitable disease The method involved considerable risks of spreading the infection, however and it was therefore not widely employed

After the discovery of the protective effect of an infection with cowpox the Jenner smallpox vaccination was introduced into Sweden in 1801

To begin with vaccination against smallpox was voluntary and the vaccination rate was low From 1816 it has been compulsory in Sweden however As from 1810 the number of smallpox cases diminished slowly but greater outbreaks still occurred After the end of an epidemic in 1874 the smallpox death rate continued to fall while the vaccination rate rose During the early 1880 s the disease was still causing more than 100 deaths a year In 1893 and 1894 there was a temporary rise, but 1895 was the first year without any case of small pox recorded in the country From 1895 to 1905 the total deaths numbered only 14 During the first two decades of this century smallpox still appeared almost every year in Sweden (Table 1) With the exception of a fairly widespread epidemic in 1917, when the infection was introduced by war invalids passing through the country from Russia to Germany, the number of smallpox cases fell slowly however The majority of cases occurred in minor outbreaks generally caused by the entry of single cases from abroad

The last outbreak in our country prior to the present one occurred in Malmo in 1932 with 13 persons affected (1) As so often before the disease came in from Russia Most of the victims belonged to the hospital personnel or were patients who had been for a short time in a waiting room simultaneously with or after the primary case During this outbreak a mass vaccination was organized in the city altogether 112 374 persons being vaccinated

Since 1932 smallpox has not occurred in Sweden On a few occasions there have been suspected cases, but in none was the diagnosis verified

Vaccination before the age of 5 is compulsory In 1960 smallpox vaccinations were performed in about 65 % of the Stockholm children and in 1965 in about 82 %

Epidemiological methods

The epidemiological investigations, which were carried out in all cases of

TABLE 1 Smallpox cases in Sweden 1900—1962

Period	Number of cases	Number of outbreaks	Number of deaths
1900—04	89	10	2
1905—09	55	6	1
1910—14	51	5	3
1915—19	244	8	23
1920—24	14	3	3
1925—29	0	0	0
1930—34	13	1	1
1935—62	0	0	0

suspected or verified variola, provided the basis for the preventive measures Data concerning the patients, their possible sources of infection and their contacts were collected in accordance with the following schedule, although the methods during the various phases of the epidemic were adapted to the prevailing epidemiological situation

I *Collection of data*

a) Questioning of the patient
b) Questioning of relatives, friends, fellow workers, etc
c) Studying of working records, diaries, appointment books, hospital records and similar documents

II *Types of data collected*

a) Concerning the patient,
   1 Name, address, profession, place of work
   2 Vaccinal state
   3 Clinical symptoms
   4 Names and addresses of possible sources of infection and time of contact
   5 Names and addresses of possible contacts and time of contact
   6 Detailed schedules of the patients' activities, such as shopping, hospital visits and travels
b) Concerning possible sources of infection or contacts,
   1 Name, address, profession, place of work
   2 Vaccinal state
   3 Clinical symptoms

III *Registration of data*

All data were recorded in a card system where each patient, suspected case or contact was given a card specially designed for these purposes (Appendix Redesigned cards according to experience during the present outbreak)

IV *Means of tracing sources of infection and contacts*

a) Personal visits
b) Telephone calls or telegrams
c) Mass media

V *Measures taken with sources of infection and contacts*

a) Questioning
b) Checking of vaccinal state
c) Vaccination, immune globulin, chemoprophylaxis
d) Isolation or surveillance
e) Taking of specimens for virological and serological diagnosis

The investigations and measures in connection with some of the smallpox patients were extensive Thus the visit of case 4 7 to the outpatient department of St Goran Hospital necessitated the perusal of some 10 000 patient re

Registration card for smallpox patients and contacts

year month day

Name ______________ Date/Birth ________ Reg no ________
Address ______________________________ Date onset ________
Post-office ______________ Telephone ________ Diagnosis ________
Occupation ______________ Place of work ________ ________
______________ Telephone ________ Risk group ________

Source of infection

Name	Reg no	Date of contact	Measures

Vaccinal history	Date	Result	Read	Measures	Date	Vaccine	Result	Read
Primary				Vacc				
Revacc				Isolation				
				Discharge				

Clinical features / Laboratory results

Clinical features		Date Onset	Date	HI	CF	CFV*	Isol	Other
Temperature	☐							
Shivering	☐							
Headache	☐							
Muscular pains	☐							
Lumbar pains	☐							
Malaise	☐							
Haemorrhage	☐							
Exanthema	☐							

Contacts

Name	Address	Telephone	Measures

Surveillance

Date	Days since 1st contact	Days since last contact	Vacc	Temp	Symptoms

* Direct CF test for poxvirus antigen in efflorescent material

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service in 1960 with positive result His last vaccination before falling ill was on May 22 1961 in Port Said According to the seaman s own statement a vesicle appeared after that vaccination

Under the Swedish Quarantine Act a person entering the country from a non European country with the exception of the United States and a few other countries shall produce an international certificate showing that he has been vaccinated against smallpox within the past three years If this certificate cannot be produced the traveller may be subjected to vaccination and/or surveillance and possibly isolation

On his arrival to Sweden L O *(1 1)* was entirely healthy and equipped with an international vaccination certificate (although not on the form approved by WHO)

On April 6 1963 13 days after his return he fell ill with fever He soon observed blisters on the face and neck As he had earlier suffered from acne he paid no particular attention to his symptoms and did not visit a doctor

The diagnosis of smallpox was based on epidemiological data clinical course high antibody titres five weeks after onset and unsuccessful vaccination on May 16 1963 with extra potent vaccine

Although it was established that the infection was brought to Sweden by the sailor L O *(1 1)* it is not clear where he was infected Epidemiological data indicate that he contracted his infection during his journey from Australia

As far as is known none of the other passengers on the planes were infected and no simultaneous cases occurred in Europe This does not exclude the possibility that an infectious passenger accompanied the plane during part of the journey but it appears more probable that L O *(1 1)* was infected at one of the intermediate landings At the time, there was smallpox in Calcutta and Karachi and an extensive epidemic was proceeding in Jakarta

Spread of smallpox in the Stockholm area (Fig 1)

Spread from the imported case

The seaman *(1 1)*, after his arrival in Sweden on March 24 1963, lived for the most part in a flat with his grand mother

During his acute period of illness he remained quietly at home for about one week and was looked after by his grand mother *(2 2)* his fiancee *(2 4)* and other relatives He was also in contact with the nurses who daily visited his grandmother

One of them, Mrs D G *(2 1)* fell ill on April 18 with high fever and muscular pains D G was admitted to the Southern Hospital in Stockholm on April 23 and died within a few hours in a disease characterized by fever thrombocytopenia and petechial haemorrhages of skin and internal organs No exanthema however, was observed

In her home D G *(2 1)* had been in contact with her husband A G *(3 1)* and his nephew K G *(3 2)*, the latter of whom became the first recognized case of the outbreak

On April 21 the seaman s grand mother *(2 2)* fell ill with high fever and exanthema for which she was treated at the Stockholm Hospital for

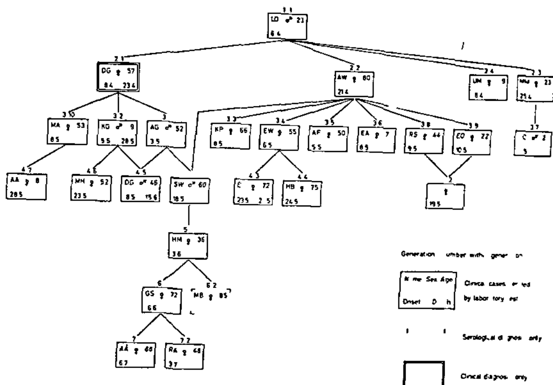


Fig 1 Evolution of the smallpox outbreak in Stockholm 1963

cords and tracing, vaccination and isolation of some 600 persons

Epidemiological investigations had also to be carried out in connection with many clinically suspected cases, which later proved not to be smallpox, since it was judged unadvisable to wait for the laboratory confirmation of the diagnosis, before such measures were taken

Origin of the outbreak

In the 1963 outbreak of smallpox in Stockholm the infection was brought to Sweden by a 23 year old seaman L O (Case *1 1* of Fig 1) Since the autumn of 1962 he had worked as a mechanic on a Swedish tanker on the Indonesian coastal trade On February 28 1963 he signed off at Derby, Australia, where he remained for some 10 days He then proceeded to Perth, arriving there on March 22 On the same day he continued by air to Stockholm with intermediate landings at Jakarta, Singapore, Rangoon Calcutta, Karachi, Teheran, Damascus, *Zurich, Dusseldorf and Copenhagen* With the exception of a day s stay in Zurich, the journey was made without a break He never left the transit area at any of the airports at which intermediate landings were made During the journey, which was made by British Airways flight 709 on March 22 and Swiss Air flight 250 from Zurich on March 24 he was not, as far as is known in contact with any ill person

L O (1 1) had been successfully vaccinated against smallpox as a child again in 1955, 1956 and 1959 successfully on one occasion), and in military

A F (*3 5*), who visited A W (*2 2*) on April 26, developed smallpox on May 5 She remained at home until May 14, being then isolated at the Stockholm Hospital for Infectious Diseases

E A (*3 6*) was a 71 year old woman without known contact with any case of smallpox However she lived in the same apartment house as A W (*2 2*), who was ill at home from April 21--27 Although the two women were unacquainted they could have met on the stairs or in the elevator

A W (*2 2*) as already mentioned, was kept at the Stockholm Hospital for Infectious Diseases from April 27 to May 7 on a diagnosis of chickenpox She infected two nurses there, R S (*3 8*) and E O (*3 9*) who worked in A W s ward The two nurses, who displayed relatively mild symptoms, continued at work during the first days of illness During that time they cared for a one year-old girl, I L (*4 2*) who had been hospitalized for whooping cough and bronchopneumonia The girl had had her primary vaccination on May 15 and at the same time was given 5 ml of immune globulin against vaccinia but nevertheless got smallpox on May 19

Another smallpox case belonging to the third generation was later discovered This was a 53 year-old layer-out M A (*3 10*) who on April 26 had laid out the corpse of D G (*2 1*) She had very mild symptoms and was not discovered until some time after her mother A A (*4 7*) fell ill with smallpox on May 28

Apart from the aforementioned four cases of smallpox three other cases developed in the fourth generation

On May 23 an already isolated nurse s assistant at the Danderyd Hospital, M H (*4 6*), fell ill although she had been successfully revaccinated within less than 24 hours of her first contact She had cared for K G (*3 2*) on the night between May 13 and 14 Her clinical symptoms were moderate

O G (*4 5*) was father of the first recognized case K G (*3 2*) and brother of A G (*3 1*) He was in Stockholm from April 28 with his wife to attend the funeral of his sister in law D G This brought him in contact with both his brother, with whom he stayed, and his son, who fell ill on May 3 and May 5 respectively On May 14, when it had been established that his son, K G (*3 2*) had smallpox O G and his wife were both given their primary vaccination O G developed a severe variola of which he died on June 15 while his wife, who presumably was subjected to the same degree of exposure as her husband did not contract smallpox

The only case in the fourth generation which gave rise to secondary cases was a 60-year-old man, S W (*4 1*), employed as a hospital worker at the Stockholm Hospital for Infectious Diseases One of his duties was to collect laundry and refuse from the ward in which were the patients A W (*2 2*) and A G (*3 1*) suspected for varicellae He fell ill on May 18 with mild clinical symptoms He went on with his work, however until he three days later visited a doctor at the hospital on account of an incipient exanthema At that time he was also transporting food from the kitchen of the Hospital for Infectious Diseases to the Norrtull Hospital at which

Infectious Diseases during the period April 27—May 7 as a case of chickenpox

The seaman's fiancee U M (2 4) fell ill around April 18 with very mild symptoms, and her sister M M (2 3), who also had been in contact with the seaman on repeated occasions fell ill a week later, on April 25 Her symptoms were more pronounced and were interpreted by the consulted physician as chickenpox, for which she was cared for at home until May 15

The first recognized case

The first recognized case in the Stockholm outbreak, K G (3 2), was a relative of the deceased visiting nurse, D G (2 1) K G (3 2), a 19-year-old bricklayer, fell ill on May 5 with pain in the small of the back, nausea, vomiting and high fever After a couple of days an exanthema appeared consisting of bright red papules, first on hands and face, but later spreading to the entire body He was admitted to the Infectious Disease Clinic of the Danderyd Hospital on May 8

During his stay in hospital, the symptoms increased and on May 12 some suspicion of smallpox arose for the first time Specimens for virological and serological examination were taken the following day

A complement fixation test against vaccinia serum with efflorescence material as antigen was positive and serological examination of serum showed a haemagglutination inhibition (HI) titre of 1/40 although the patient had never been vaccinated against smallpox

Further development of the outbreak

The epidemiological investigation which was started as soon as the preliminary virological results were available on the evening of May 13, revealed that K G had not recently been abroad nor, as far as is known, had been in contact with anyone who had been abroad It also appeared that his uncle A G (3 1) had been admitted to the Stockholm Hospital for Infectious Diseases on May 7 owing to fever and exanthema of unknown etiology and that his aunt, D G (2 1) had died at the Southern Hospital of an obscure haemorrhagic disease

In the subsequent epidemiological investigation it soon appeared that D G (2 1), in her work as home visiting nurse, had looked after A W (2 2) and so come into contact with the sea man L O (1 1) Through him the trail led to his fiance U M (2 4) and her sister M M (2 3) M M s fiance, K C (3 7), who had fallen ill on May 11 with headache and fever, was ad mitted to the Stockholm Hospital for Infectious Diseases on May 15

A W (2 2), despite her relatively isolated life, gave rise to several secondary cases On May 8 K P (3 3), a nurse who had visited A W on April 24, fell ill with mild smallpox, while the daughter-in law, E W (3 4) who had nursed her on April 23 and 26 came down on May 6 E W (3 4) was ad mitted to the Southern Hospital on May 9 and on account of fever and pains in the back, she was suspected to have pyelitis While at the Southern Hospital she infected two of her four room mates, E T (4 3) and H B (4 4)

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there were some 300 female mental patients

On June 16 a patient, G S (*6 1*) in Ward 3 of the Norrtull Hospital devel oped smallpox However, smallpox was not suspected until June 22 The epidemiological investigation revealed the source of infection to be a woman, H M (*5 1*), who had worked in the kitchen of Ward 3 and had fallen ill on June 3 with fever and later eruptions on the arms Apparently this woman had been in contact with S W (*4 1*), probably on May 18 She had been successfully vaccinated against smallpox on May 17 During her illness she was seen only by a nurse who interpreted her disease as a postvaccinal reaction Obviously the incubation period was too long for vaccinia, however

Rigorous isolation measure were taken at the Norrtull Hospital All personnel and patients were vaccinated and/or given immune globulin against vaccinia Another three cases of smallpox occurred among the isolated patients of Ward 3, M B (*6 2*), A Å (*7 1*) and R A (*7 2*)

No other cases were observed, and the Stockholm area was declared free from smallpox on August 5

Epidemiology of hospital infections

The combating of the outbreak at the affected hospitals offered problems which in many respects differed and were in some cases greater than those in the community at large This was accen tuated by the fact that, at the time when the first recognized case was diagnosed, the outbreak had to a large extent con centrated to the hospitals Therefore an account is given of the spread of the infection and of the measures taken at each hospital, since - differing in size, type and organization — each hospital had to tackle the problems in its own way

The Southern Hospital

The Southern Hospital is one of the largest general hospitals in Stockholm with about 1,600 beds and a personnel of approximately 2,100 Most wards are situated in a long 9 storey wing The after-care and pediatric services are located in separate buildings, connected with the main building by open subways The various outpatient departments, laboratories, etc , are situated in a wing parallel to the 9 storey wing and connected with it by three open corridors on each floor The admission and emergency departments are located on the ground floor in premises between the two wings

When the first recognized smallpox case, K G (*3 2*), was discovered on May 13, it was soon established that his aunt, D G (*2 1*), had been admitted to the Southern Hospital on April 23 On admission she had been in a very poor condition and she was not removed from the admission department She died there within a few hours She was in contact with only a few other patients and members of the personnel She was suffering from a severe hemorrhagic condition Smallpox was not suspected The body was taken to the post mortem department on a covered bier and

autopsy was performed on April 24 The diagnosis was thrombocytopenia haemorrhagica cum diathesis

On re evaluation of the records from this patient it was concluded that the cause of death was purpura variolosa

On May 15 an inquiry was made for the contacts of D G 3 weeks earlier at the admission and post mortem departments No suspect cases of smallpox were found As the longest incubation period had already elapsed at that time it was believed that no secondary cases had occurred It proved later however that M A (*3 10*), the layer-out of the corpse had been ill with mild symptoms Not belonging to the hospital personnel she was overlooked at the investigation and remained undetected until June 6 when her mother A A (*4 7*) was found to have smallpox

D G s husband A G (*3 1*), had fallen ill on May 3 with fever headache and pain in the back He was seen in the admission department of the hospital on the same day As he was only moderately ill with indistinct symptoms he was sent home On May 7 he again appeared at the admission department now with a rash of doubtful origin Within a few hours he was transferred to and kept at the Stockholm Hospital for Infectious Diseases His disease was later found to be smallpox

E W (*3 4*) daughter in law of A W (*2 2*) came to the admission department on May 9 She had fallen ill on May 6 with high fever and pain in the back After about 4 hours she was transferred to a medical ward situated at the far end of the hospital Before being taken to the ward on a litter she was given a wash in the central baths She spent five days in this 32 bed ward in a room for four patients She stayed in bed but made regular visits to the common dressing room She and her room mates received several visits On May 10 she was carried in her bed to the E C G laboratory in the centre of the hospital but was not removed from her bed No other visits were paid outside the ward Two days after her admission a rash was observed This was believed to have been caused by the sulpha treatment, given for the suspected pyelitis On May 14 she was transferred to a dermatologic ward on account of her exanthema This ward is situated at the other end of the hospital on the top floor To get there, she was carried in her bed through most of the hospital She stayed here for almost two days in a room for two patients

On May 15 smallpox was *suspected* It was found that she had had contact with A W (*2 2*) She was immediately transferred to the Stockholm Hospital for Infectious Diseases E W gave rise to two secondary cases E T (*4 3*) and M B (*4 4*), who had been her room mates from May 9 to 14

During their visits on May 3 7 and 9, A G (*3 1*) and E W (*3 4*) had been in the admission department which was at the same time visited by many other patients It was found that they might have been in contact with some 150 patients during those three days About half of these patients had later been hospitalized in the Southern Hospital The others had been sent home or to other hospitals Efforts were made to trace these contacts for vaccination and

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15 Particular attention was paid to symptoms and signs among patients and personnel which could be caused by smallpox One of the physicians in the hospital was appointed to examine all suspect cases All suspect symptoms were to be reported to him

16 Directions were issued concerning treatment of objects that might be contaminated, such as laundry, refuse, china etc, as well as concerning personal hygiene and behaviour

17 The personnel was informed of the emergency measures at meetings in the central lecture hall and by means of printed circulars and announcements on the local radio

18 All remaining patients in the two wards in which E W (3 4) had been were eventually transferred to isolation establishments outside the hospital This was done in order to permit final disinfection of these wards prior to the reopening of the hospital

19 As no secondary cases appeared among the personnel or among the remaining patients the isolation of the hospital was abandoned on June 1, i e on the 17th day after the removal of E W (3 4)

Vaccinal state at the hospital when smallpox was discovered

Reliable information of previous vaccinations was obtained from 1 114 employees and 767 adult patients The figures in per cent are shown in Table 2

TABLE 2 Vaccinal state of personnel and patients at the Southern Hospital on May 15 1963

Last successful vaccination	Personnel 1 114	Patients 767
1962—April 1963	45 1 %	15 4 % (1953—April 1963)
1953—1961	20 9 %	
prior to 1953	31 7 %	79 1 %
not vaccinated	2 3 %	5 5 %

Results of vaccination of personnel and patients

Altogether 2 069 out of the 2 082 members of the personnel were vaccinated on one or more occasions Eight of the remainder had been vaccinated successfully between 1962 and April 1963, and 5 were not vaccinated because of medical reasons Successful result was obtained in 95 3 % while the rest remained negative on repeated vaccinations Most of the latter individuals had been successfully vaccinated in 1962—April 1963 Among 1 190 nurses and aids, 123 (10 3 %) were off duty for an average period of 3 7 days because of reported reactions to the vaccination

Of the adult patients, 822 out of 942 were vaccinated 95 8 % successfully Fortyone per cent of the patients were more than 60 years of age The majority of the patients tolerated the vaccination very well but severe reactions occurred in quite a few No postvaccinal complications came to our knowledge however

Of the 45 patients in the two wards in which E W (3 4) had been only four had initially a satisfactory vaccination Thirtyfour had been vaccinated more than 10 years previously, the majority in infancy three had never been vaccinated and four did not know whether they had been vaccinated or

isolation None of these contacts developed smallpox

Soon after the diagnosis of the first recognized case (3 2) it was thus realized that the Southern Hospital had been seriously exposed to smallpox

Measures taken

Extensive measures were immediately taken to limit the spread of the infection within the hospital and to prevent its dissemination into the community These measures were principally as follows

1 Immediate strict isolation of the admission department and of the wards in which the smallpox cases had been taken care of

2 Disinfection of the rooms in which the smallpox patients had been

3 Prohibition of all outside visits to inlying patients

4 Admission to and discharge from the other wards in the hospital was stopped This came into force on May 17

5 Ambulant salesmen, librarians, etc were forbidden to circulate within the hospital as were as far as possible certain general personnel such as physiotherapists

6 All traffic within the hospital was reduced as much as possible The patients had to stay within their wards and visits to X ray and other departments were allowed only on strict indications

7 Contacts of A G (3 1) and E W (3 4) within the hospital, among patients that had left the hospital, and among visitors were traced, vaccinated and isolated, if necessary

8 An inquiry to find out the current vaccinal state of the hospital personnel and the hospitalized patients was circulated on May 16

9 All members of the personnel were vaccinated on May 15—17 A compulsory check of the result of vaccination was organized on May 21 All persons with a negative or doubtful result were revaccinated with an extra potent vaccine In many cases the vaccination had to be repeated several times

10 Isolation wards were organized within the hospital All members of the personnel who were considered to have been exposed and whose vaccinal state at the time of exposure was not satisfactory, altogether some 100 persons, were isolated

11 All remaining patients in the hospital were vaccinated, most of them on May 18 In case of medical contraindication vaccinia immune globulin was given instead

12 Vaccination of relatives of the personnel was started on May 17 in a separate building within the hospital area

13 The four room mates of E W (3 4) were transferred to the Clinic for Infectious Diseases of the Danderyd Hospital on May 18

14 The hospital s extensive outpatient service was almost entirely suspended from May 20 To minimize the disadvantage of this rather drastic measure, all patients who showed up were given an opportunity of talking to a doctor at the entrance of the hospital Most patients could without harm be advised to come back later Those who needed medical treatment were admitted to the outpatient department, where they were also offered vaccination

TABLE 3 Stockholm Hospital for Infectious Diseases Vaccinal state of contacts among personnel and medical students not vaccinated during the last 1 5 years before contact

Successful vaccination before contact			Successful revaccination after contact		
Last successful vaccination	Total number of persons	Vaccinated more than once	On day of contact	One day after contact	Contact one day only
1961	7	7	1	1	3
1960	3	3	—	—	1
1958—59	4	3	1	—	3
1957—53	6	4	[3]0	—	5
1952—48	[1]8	4	[3]0	—	3
1947—43	5	[2]0	—	2	4
1942 or earlier	5	[2]0	—	—	2
None	2	0	—		1
Total	*40*	*21*	2	3	22

[1] Two of these individuals contracted smallpox
[2] Three individuals had been revaccinated without success
[3] One individual got immune globulin whereas a simultaneously performed vaccination did not take

passed through an entrance room simultaneously with another patient who had only been vaccinated as a child, but no infection was transmitted When the first patient A W (2 2) was discharged on May 7 only a routine cleaning of her room was carried out thus her mattress was not disinfected The next patient admitted to her room had a satisfactory vaccinal state however

A great number of personnel and medical students had visited the rooms of the undiagnosed smallpox patients and some of them had an unsatisfactory vaccinal state (Table 3) Many had been in close contact with the patients Two nurse s assistants contracted mild clinically unrecognized smallpox (E S *3 8* and L O *3 9*) one of them had been vaccinated as late as 1962, the other not since 1950 Apparently they spread the infection to a one year old patient admitted to their ward for pertussis (I L *4 2*)

Indirect contact probably caused the smallpox of the hospital worker S W (*4 1*) who transported laundry and refuse from the observation wards, he had not been vaccinated since 1949 Assisting also in the transport of food from the kitchen of the *Hospital for Infectious Diseases* to the Norrtull Hospital he transmitted the infection to a nurse s assistant (H M *5 1*) at this hospital, thereby causing a nosocomial outbreak

Once the first diagnosis of smallpox had been established measures were taken to prevent the spread of the infection within the hospital At the same

not Forty three of the 45 were vaccinated on May 15 successfully in all but one *In spite of the obvious infection risks* and the poor immunity, only two of these patients, both room mates of E W (*3 4*) developed smallpox Both were vaccinated about 70 years ago The *two other room mates* escaped infection although they had a similar vaccinal state

Among the personnel, 40 persons with an unsatisfactory vaccinal state were found *to have been in close contact with the three smallpox patients All had been in the* same room as the patients and the majority had been in direct contact with them in conjunction with bed making, *examination, collection of samples, au*topsy, etc The vaccinal state of this group was as follows Six had been vaccinated in 1960—1961, six in 1953—1959, ten in 1943—1952 and 17 prior to 1943, while one had not been vaccinated at all All these people were successfully vaccinated on May 14—17, and isolated None developed smallpox

Comments

The Southern Hospital, a large general *hospital with busy internal communica*tions, must be considered to have been seriously exposed to smallpox A large number of members of the personnel and patients had been in more or less close contact with smallpox cases The initial vaccinal state of the personnel was fairly good, as 45 % *had been vaccinated* within approximately the last year The patients, on the other hand, were poorly protected A rapid and effective vaccination program was accomplished and isolations of contacts and other measures were taken *as soon as possible All this* probably contributed to the fortunate outcome of this serious incident Of the many exposed, only three developed clinical signs of smallpox Two of them had for four days *been room mates of* E W (*3 4*), the third had taken care of the dead body of D G (*2 1*)

The various emergency measures naturally involved a major interference with the normal hospital activities and with the public medical service It was *feared that the almost complete closing of all services of one of the largest hos*pitals in the area might be of serious consequences to the public It turned out, however, that this situation could be *rather easily managed It was, of course,* urgent that the period of closure should be as short as possible without neglect of the security requirements from epidemiological points of view The hospital was closed until May 31 and was reopened to the full extent on June 1, *after disinfection and thorough cleaning* of all contaminated departments and wards (see chapter I page 33)

The Hospital for Infectious Diseases in Stockholm

When the smallpox case K G (*3 2*) was diagnosed *on May 13 it was discovered* that two similar cases had been hospitalized for chickenpox at the Stockholm Hospital for Infections Diseases for some time, A W (*2 2*) from April 27 to May 7 and A G (*3 1*) since May 7 Both patients had been cared for in a *modern observation ward with good iso*lation facilities, where they had no op*portunities for direct contact with other* patients One of them had, however,

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3 Vaccination of relatives of the personnel

4 Sixty members of the hospital personnel whose immunity was regarded as unsatisfactory (not successfully vaccinated since January 1962) and who might have been in contact with K G (*3 2*) were isolated in the hospital or in an isolation establishment

5 The patients who had been in contact with K G during his visit to the X ray Department were isolated, altogether 18 persons

6 Ward 5 was subsequently used for isolation of suspect smallpox cases and for the co-patients of K G who might have been exposed The ward was strictly isolated from the hospital and the personnel on duty was accommodated in a neighbouring evacuated ward

7 A number of wards adjoining the Infectious Disease Clinic were evacuated to provide space for the isolation of exposed persons or for individual with postvaccinal complications

8 Prohibition of all outside visits to inlying patients

9 As far as possible patients who had not been exposed to infection were discharged and admissions were curtailed

10 Disinfection of laundry refuse etc organized

From May 18 to 23 eleven patients were transferred from the Southern Hospital for isolation Four of them exhibited suspect symptoms of variola on May 23 In two the symptoms proved to be postvaccinal reactions, while the other two E T (*4 3*) and H B (*4 4*) who had been room mates with E W (*3 4*) at the Southern Hospital developed smallpox E T died on May 27 while H B survived H B was transferred on May 31 to the Stockholm Hospital for Infectious Diseases During their time at Danderyd Hospital these two smallpox cases had been effectively isolated and did not cause any secondary case

On May 23 the nurse s assistant M H (*4 6*), who had cared for K G (*3 2*) on the night between May 13 and 14, fell ill with smallpox She had been vaccinated 50 years previously She was revaccinated successfully on May 14 (within 24 hours of infection) and was thereafter isolated After falling ill she was transferred to the smallpox pavilion of the Stockholm Hospital for Infectious Diseases

Thus at Danderyd Hospital, too, the smallpox outbreak necessitated extensive changes of organization

St Goran Hospital

On May 28 the 84 year old woman A A (*4 7*) fell ill feeling tired and with a headache She lived with her daughter M A (*3 10*), who on April 26 had laid out the corpse of D G (*2 1*) at the Southern Hospital and 12 days later had developed a slight illness A few days after falling ill, A A developed an exanthema on the back of the neck, in due course spreading over the body

A A had for several years been treated for pernicious anaemia and on June 6 she visited the medical outpatient department of St Goran Hospital accompanied by her daughter for a routine check up She arrived at the hospital at 8 30 a m Before leaving for the hospi

time the hospital had to be reorganized for the care of smallpox patients from the entire Stockholm area as described in detail in chapter III, page 66

Measures taken

1 Isolation of the observation ward in which smallpox patients were kept Together with two other isolated observation wards it was subsequently used for the care of suspected cases of smallpox Organization of a separate pavilion for the treatment of smallpox cases

2 Isolation and vaccination of patients who had been in the observation ward at the same time as the smallpox cases Some were in addition given vaccinia immune globulin

3 All other patients of the hospital were vaccinated provided there was no distinct medical contraindication Immune globulin was instead given in some cases

4 Prohibition of visits to inlying patients

5 All personnel including medical students vaccinated on May 14—17 The results of vaccination were checked and revaccination was repeated until successful

6 All employees and students who might have been exposed and whose vaccinal state was suspected to be unsatisfactory were isolated most of them within the hospital

7 Vaccination of relatives of the personnel

8 Particular attention was paid to symptoms among patients and staff which could be caused by smallpox suspected symptoms were to be reported

9 Disinfection of laundry, refuse etc. organized (see chapter III, page 67)

The Clinic for Infectious Diseases of the Danderyd Hospital

The first recognized case, K G (*3 2*), was admitted on May 8 to Ward 5 of the Clinic for Infectious Diseases of the Danderyd Hospital because of fever and a suspect exanthema This ward, which occupies one wing of the clinic was built to allow for the isolation of smallpox cases It consists of 8 isolation rooms with sluice, all with separate ventilation K G (*3 2*) left the isolation room only on one occasion, on May 9, when he was X rayed He had to wait for several hours in the corridor outside the X ray department of the Infectious Disease Clinic along which patients and personnel were passing

Due to clinical suspicion of variola specimens were taken on May 13 for virological and serological examination, preliminarily yielding positive results that same evening The diagnosis was subsequently verified by isolation of virus On May 14 K G was transferred to the smallpox pavilion that had been organized at the Stockholm Hospital for Infectious Diseases

Measures taken

1 On May 14 some 90 % of the personnel of the hospital were vaccinated, the remainder on the next few days

2 All patients in the Infectious Disease Clinic were vaccinated insofar as their condition allowed immune globulin was also given to a limited extent

3 Vaccination of relatives of the personnel

4 Sixty members of the hospital personnel whose immunity was regarded as unsatisfactory (not successfully vaccinated since January 1962) and who might have been in contact with K G (*3 2*) were isolated in the hospital or in an isolation establishment

5 The patients who had been in contact with K G during his visit to the X ray Department were isolated al together 18 persons

6 Ward 5 was subsequently used for isolation of suspect smallpox cases and for the co-patients of K G who might have been exposed The ward was strictly isolated from the hospital and the personnel on duty was accommodated in a neighbouring evacuated ward

7 A number of wards adjoining the Infectious Disease Clinic were evacuated to provide space for the isolation of exposed persons or for individuals with postvaccinal complications

8 Prohibition of all outside visits to inlying patients

9 As far as possible patients who had not been exposed to infection were discharged and admissions were curtailed

10 Disinfection of laundry refuse etc organized

From May 18 to 23 eleven patients were transferred from the Southern Hospital for isolation Four of them exhibited suspect symptoms of variola on May 23 In two the symptoms proved to be postvaccinal reactions while the other two E T (*4 3*) and H B (*4 4*), who had been room mates with E W (*3 4*) at the Southern Hospital developed smallpox E T died on May 27, while H B survived H B was transferred on May 31 to the Stockholm Hospital for Infectious Diseases During their time at Danderyd Hospital these two smallpox cases had been effectively isolated and did not cause any secondary case

On May 23 the nurse s assistant M H (*4 6*) who had cared for K G (*3 2*), on the night between May 13 and 14, fell ill with smallpox She had been vaccinated 50 years previously She was revaccinated successfully on May 14 (within 24 hours of infection) and was thereafter isolated After falling ill she was transferred to the smallpox pavilion of the Stockholm Hospital for Infectious Diseases

Thus at Danderyd Hospital too the smallpox outbreak necessitated extensive changes of organization

St Goran Hospital

On May 28 the 84 year-old woman A A (*4 7*) fell ill feeling tired and with a headache She lived with her daughter, M A (*3 10*) who on April 26 had laid out the corpse of D G (*2 1*) at the Southern Hospital and 12 days later had developed a slight illness A few days after falling ill A A developed an exanthema on the back of the neck in due course spreading over the body

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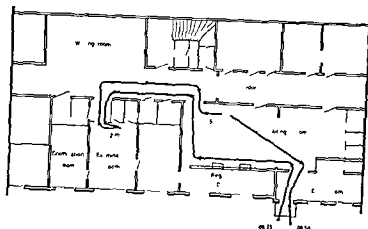


Fig 2 Route of case 4 7 in St Goran outpatient department of surgery and medicine

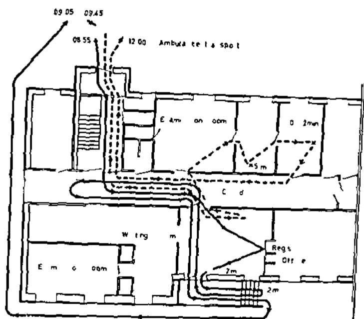


Fig 3 Route of case 4 7 in St Goran outpatient department of dermatology

tal her daughter had informed a nurse at the medical outpatient department that her mother had a rash which might possibly be smallpox This announcement, being not uncommon at the time, was unfortunately not taken seriously by the nurse, and she did not act upon it A schedule of A A s moves in the hospital is seen in Figs 2 and 3 The nurse noticed her skin lesions and immediately sent her to the dermatological outpatient department of the same hospital When A A arrived there she was told to wait outside in the grounds until she was called for Trying to find the rear exit into the grounds she passed several times through the waiting room before finally going out through the main entrance About one hour later a dermatologist came out to see her The doctor thought that the visible skin changes could hardly be variola and helped the woman back into the waiting room After some further delay A A was taken into an examination room where she undressed and was examined

When the dermatologist now saw her exanthema in full smallpox was suspected. After the head physician had confirmed this diagnosis A A was at noon time transferred to the Stockholm Hospital for Infectious Diseases

A A's visit to the St Goran Hospital took place during the rush hours at the outpatient departments Since the medical department has a waiting room in common with the surgical and diabetes departments A A was considered to have been in contact with a large number of other visitors On arrival at the Hospital for Infectious Diseases she was found to have efflorescences also in the mouth and pharynx She was consequently considered to be very infectious Rigid precautions were therefore taken

Measures taken

All personnel of the outpatient departments were revaccinated on the afternoon of June 6 Out of the 61 personnel who had been in the outpatient departments concerned at least 17 had been vaccinated since the outbreak started in Stockholm and another 22 had undergone their last successful vaccination in 1962

An extensive action to trace all patients who had visited the outpatient departments on June 6 was then started After a perusal of some 10 000 patient cards it was found that about 600 had visited the hospital on that day The number of persons who had been in the departments was assumed to be greater however as many patients had been accompanied by a relative Many hospital employees had also passed through the departments

Each patient was requested by telephone telegram or a personal visit to come to the hospital for vaccination as soon as possible Summons were also issued on June 7 through mass media On June 7, 334 persons presented for vaccination 130 on June 8 and another 145 on the following days altogether 609 In addition 30 out patients had been admitted to the hospital They were all revaccinated

In some cases vaccinia immune globulin was used in addition to or instead of vaccination

All persons who had visited the outpatient departments at the same time as the smallpox case or within 4 hours after she had left were considered to have been exposed Persons with satisfactory vaccinal state, i e successfully vaccinated since Jan 1, 1962 were placed under surveillance The others were isolated during the period of day 8—16 after exposure

No action was taken in respect of persons who visited the outpatient departments more than 4 hours after the smallpox case had left but on the same day, provided that their vaccinal state was satisfactory Those who were not considered to have sufficient immunity, were placed under surveillance if they had been revaccinated on one of the first days following their visit to the hospital — the remainder were isolated and vaccinated

Altogether some 500 persons hospital personnel included were isolated and 150 were placed under surveillance The

remaining 100 or so were released without action

These extensive isolation measures naturally caused difficulties Some of the patients had been admitted to the hospital via the outpatient departments and therefore constituted a potential risk to the hospital Many of the other out patients were in need of medical attention and could therefore not be isolated on a merely provisional basis

On the basis of medical and social criteria, the material had to be grouped according to the nursing resources of the various hospitals and isolation establishments Thus contacts who required to be hospitalized were allocated to one of the infectious disease hospitals The contacts who merely required medical attention were placed in convalescent homes, while healthy persons were isolated in schools or barracks

It may be mentioned that the measures were met with a great understanding and cooperativeness on the part of all concerned In only one case was police assistance required

No secondary case occurred in the isolation or surveillance groups

Norrtull Hospital

The last three generations of smallpox cases in the Stockholm outbreak occurred at the Norrtull Hospital with some 300 female mental patients

On June 16 a 72 year-old woman, G S (*6 1*), a patient in Ward 3, fell ill with high fever and a rash in the right axilla She was moved on the same day into a single room in the ward After a few days a generalized variola-like exanthema appeared and on June 22 she was transferred to the Stockholm Hospital for Infectious Diseases as a suspect case of smallpox

The Norrtull Hospital comprises a number of old three-storey buildings As it has no kitchen for the patients, food was daily brought in containers from the kitchen of the Stockholm Hospital for Infectious Diseases The infection was probably transmitted to the Norrtull Hospital by a hospital worker (*4 1*), in charge of these transports He had fallen ill on May 18 with mild smallpox but continued with his work during the first days of his illness

The containers were to be left on carriages placed outside the locked door of each ward, where they were then collected by the ward's own personnel One day in the middle of May, possibly on May 18, the lift was out of order and the hospital worker therefore had to change his routine He may on that occasion have chatted with the nurse s assistant H M (*5 1*), but in such case probably outdoors

H M (*5 1*), who was successfully vaccinated against smallpox on May 17 fell ill on June 3 with nausea, vomiting and headache She nevertheless continued at work for the first few days but was sicklisted on June 6 and isolated on June 23 as suspected source of infection of case *6 1*

H M (*5 1*) apparently also infected M B (*6 2*) who had a high HI antibody titre already on the day of vaccination This patient exhibited no clinical symptoms, however

G S (*6 1*) gave rise to two mild secondary cases, A Å (*7 1*) and R A

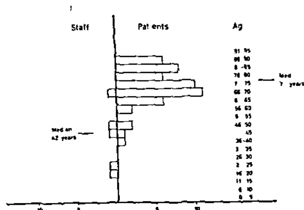


Fig 4 Age distribution of contacts at the Norrtull Hospital

(7 2), both of whom also were patients in Ward 3

When smallpox was recognized on the 22nd of June 280 patients were staying at the Norrtull Hospital 53 of whom were patients in Ward 3 None of the patients had a satisfactory immunity and only 30 of them had scars from earlier vaccinations All were considered as exposed

There had been 105 people working in the hospital on some occasion during May 18—June 22 The vaccinal state of the personnel was relatively good, as extensive vaccinations had been organized in 1962 and again in May 1963 on account of the present outbreak Altogether 29 employees had worked in or otherwise been in contact with Ward 3 Five of them had not been successfully vaccinated since January 1962 and were therefore isolated

Measures taken

1 *Immuno and chemoprophylaxis*

All personnel including those vaccinated in May 1963 were revaccinated altogether 120 persons as were some 100 relatives of the personnel

Vaccination of the patients was considered to involve serious risks for complications as the average age was high (Fig 4) Many patients were in a poor condition and, furthermore, were constantly scratching themselves

Thirthy-one of the 51 patients in Ward 3 were vaccinated on June 23 (Fig 5) At the same time they were given 5 ml of immune globulin against vaccinia The remaining 20 patients who were believed not to withstand vaccination were instead given 10 ml of immune globulin

In the next few days the patients of the other wards were vaccinated and/or given immune globulin Altogether 261 of the 278 remaining patients were successfully vaccinated Immune globulin was given to 135 of the hospital patients

At ward 3 25 of the 50 patients were in addition to immune prophylaxis also given 3 g Methisazone twice daily during June 25—28 Many of them suffered severely from nausea and vomiting One of the smallpox cases occurred in this group

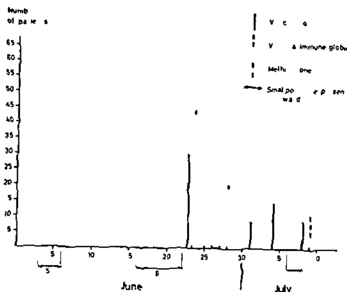


Fig 5 Immuno and chemo-prophylaxis of patients at Ward 3 of the Norrtull Hosp tal

2 *Isolation*

The hospital as a whole was turned into an isolation hospital and contact with the outside was allowed only through well vaccinated personnel Ward 3 was isolated from the remainder of the hospi tal and all its doors except a direct exit to the grounds were locked and door chinks and ventilators were sealed For change of clothing and baths all per sonnel of the ward passed through an evacuated ward

The isolation measures were facilitated by the fact that being a mental hospital communication with the outside as well as within the hospital was normally somewhat restricted

3 *Search for further cases*

In order to detect any further cases of smallpox at an early stage a thorough examination was made of all patients in the hospital every day The temperatures of all patients in Ward 3 were taken twice a day

To detect subclinical smallpox cases all patients were examined serologically Pared samples were collected from 269 out of the 280 patients, from the remain ing on one occasion only

A notable feature of the smallpox out break at the Norrtull Hospital like at the Southern Hospital is the low mor bidity despite the originally very poor vaccinal state of the exposed patients (2)

The favourable outcome may be attrib uted to several circumstances G S (6 1) for instance had relatively brief contact with her co patients before she was transferred to a single room It was also possible to seal off the ward effectively from other parts of the hospi tal The relatively satisfactory vaccinal state of the personnel and the extensive prophylactic treatment of the patients may also have been of importance

Finally it was notable that despite high age poor vaccinal state and poor physical condition the patients came through the vaccinations without any real complications All smallpox pa tients recovered although one of them had not been expected even to tolerate vaccination

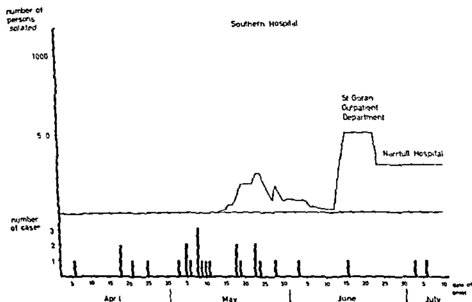


Fig 6 Number of contacts kept in isolation during various phases of the outbreak
——— Isolated contacts
Patients retained at the Southern Hospital known contacts excluded

Preventive measures

The first recognized case (3 2) belonged to the third generation of the outbreak and had been ill for 8 days when his smallpox was diagnosed on the evening of May 13 1963 As appears from Fig 1 on page 12 all patients of the third generation had fallen ill by that time The outbreak had thus become fairly widespread before preventive measures could be taken

The methods adopted were on the same general lines as in other European smallpox epidemics in recent years By means of epidemiological investigations attempts were made to trace the sources of infection and the contacts of known cases of smallpox and thereafter by immune prophylaxis and isolation to prevent the further spread of the disease

Vaccination was also immediately prescribed for various occupational groups such as hospital personnel which for different reasons were thought to run a greater risk of coming into contact with smallpox cases than the remainder of the population

Isolation of contacts

A main principle in the combating of the outbreak was that all recognized contacts with unsatisfactory immunity should be isolated in a hospital or provisional isolation establishment and that isolation at home or surveillance should be allowed only if the risk for infection was considered very small Immediately after the first recognized case had been diagnosed a number of isolation units were therefore organized at which contacts

could be received Facilities were also arranged for the isolation of patients suspected for smallpox at the two infectious disease hospitals in the area In addition most persons who had had long or close contact with a smallpox case were isolated at these hospitals, while more superficial contacts were placed in the provisional isolation establishments As soon as an isolated person exhibited symptoms which might be signs of variola, he was transferred to an infectious disease hospital

Contacts, when encountered, were immediately vaccinated and those not successfully vaccinated since January 1962 were isolated from the 7th—8th day after the first possible exposure until the 16th day after the last contact During the later stages of the outbreak close contacts which had been vaccinated in 1962 but not in 1963 were placed under surveillance for a similar period of time Family members of close contacts were vaccinated, if possible Altogether 87 close contacts in the community were isolated The number of hospital contacts isolated was much greater (Fig 6)

Epidemiologically indicated vaccinations and voluntary mass vaccination

Announcements concerning the importance of smallpox vaccination in the press, radio and television, met with a quick response from the public both in Stockholm and in other parts of the country The authorities, hospitals and other institutions concerned were immediately hard pressed with inquiries of different kinds An information centre was therefore set up (see chapter I page 41)

Physicians who performed vaccinations were soon overwhelmed with requests both from private individuals and from business and other organizations A number of vaccination centres were therefore established in order to provide a better service for the public

Certain groups of people, where vaccination complications might be expected, such as pregnant women and eczematic patients were referred to a team of specialists established at the Karolinska Hospital This team also had at its disposal immune globulin against vaccinia

At the Stockholm Hospital for Infectious Diseases an outpatient department was established for care of persons with postvaccinal serious reactions or complications Furthermore several wards were reserved for patients with such complications

The National Board of Health issued advice to the physicians about vaccination and contra indication to vaccination In a nationwide circular advice was also given to the public concerning the care of postvaccinal lesions

At an early stage preparations were made for mass vaccination should this prove necessary The National Board of Health drew up a plan for mass vaccination including arrangements for rapid training of medical students as vaccinators, detailed plans for assistance of vaccinators, premises, supplies etc The army were to place secretarial personnel at the disposal of the vaccination groups

The situation did not become so serious that mass vaccination was considered necessary on epidemiological grounds There was, however, a very

great interest in vaccination Many had to procure a vaccination certificate for travel abroad others wished to ensure their immunity

Vaccinations for direct control of the outbreak were performed by patrols organized by the Stockholm Board of Public Health Each patrol consisted of a physician and four public health inspectors Working in provisional premises at each place they vaccinated person who had been in the vicinity of smallpox cases Some of these vaccinations involved large numbers of people e g 700 of the personnel of the Stockholm City Health Insurance Service where two members of the personnel (*2 3* and *2 4*) had contracted small pox Another example was the vaccination of some 2,000 persons in the Nybohov residential area where two smallpox patients were resident Being on daily duty these vaccination groups could institute prophylactic measures at an early stage

At hospitals and laboratories which had contact with smallpox patients or samples all the personnel were vaccinated The vaccinal state of hospital employees prior to the outbreak was fairly satisfactory since vaccinations had been made on a large scale in 1962 on account of the smallpox outbreaks in Europe in 1961 and 1962 In 1962 the National Board of Health prescribed that hospital personnel should be revaccinated every third year and certain exposed groups every year

At the beginning of the outbreak relatives of the employees in exposed hospitals and laboratories were also offered vaccination and inlying patients were vaccinated to a large extent Altogether 12 000 patients and personnel were vaccinated at these hospitals

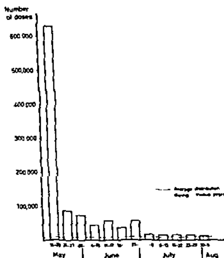


Fig 7 Doses of smallpox vaccine distributed May 14—August 5 1963

Vaccinations were also prescribed at an early stage for all personnel of hospitals which had not been visited by a smallpox case

The National Bacteriological Laboratory — the only producer and supplier of smallpox vaccine in the country — distributed some 1 050,000 nominal doses of smallpox vaccine between May 13 the date on which the first case was diagnosed and August 5, when Stockholm was declared free from infection Nearly half *a million of these doses* (453 241) went to the Greater Stockholm area The yearly output of vaccine in periods without smallpox in Europe had been, on an average 420 000 doses

The demand for vaccine rose sharply immediately after the announcement of the first diagnosis and in the following

TABLE 4 Vaccinations in greater Stockholm area May 14—July 31, 1963

Contacts in the community	12,000
Hospitals with variola contact	
Hospital personnel	6,200
Relatives of personnel	4 400
Patients	2 100
Personnel at other hospitals	11,000
Total number of epidemiologically indicated vaccinations	35 700
Total number of vaccinations according to inquiry	420 000

week more than 600,000 doses were distributed (see Fig 7)

Egg vaccines of two potencies were *delivered, one containing* $10^{7.4}$ *TCID*$_{50}$ (monkey kidney) per ml and an extra potent one with $10^{8.2}$ TCID$_{50}$ per ml recommended for revaccination of persons, who had been vaccinated within 5 years *(3, 4) (MF 53/54 1962)*

To get an idea of the number of persons actually vaccinated, the Epidemiological Department in November 1963 made an inquiry among all physicians of the Greater Stockholm area as to the number of vaccinations they had performed during the period May 14—July 31 The questionnaire was sent to 2,305 physicians who were asked to give an exact or estimated figure of the number of persons vaccinated It was also sent to military units, schools and government *departments within the area* so as to obtain a complete coverage

The questionnaire was answered by 2,121 physicians, 1,270 of whom reported 364,787 persons vaccinated

As regards physicians who did not answer the questionnaire, an estimate was made of the probable number of vaccinations based on the quantities of vaccine taken by them The calculation was based on the figures of a control group selected at random from *among the answers*

The number of vaccinated persons in the Greater Stockholm area during the smallpox epidemic was calculated in this way to be around 420,000 The relation *between vaccinations of contacts in the* community and the number of vaccinated hospital personnel is shown in Table 4

Thus during a few weeks after May 14 about *half* a *million people* were vaccinated in the Greater Stockholm area This placed a heavy burden on outpatient as well as hospital services, which was a definite drawback in view of the heavy *demands made on certain hospi*tals and on the public health services at that stage of the outbreak

In the long run, however it appears as an advantage that the vaccinal state of the popula*tion in the area was greatly* improved for years ahead

Disinfection

1 *Homes of patients suspected for smallpox*

Disinfection was carried out by sanitary inspectors from the Stockholm Board of Public Health

The patient's underwear, bedding and towels were collected in linen sacks which were sprayed on the outside with 2 % chloramine before transportation for disinfection in a formalin disinfection oven His bathroom was washed with 5 % chloramine The whole apartment was finally treated by formalin fumigation for 24 hours

2 *Hospitals*

At the observation wards of the infectious disease hospitals where patients suspected for smallpox were isolated before the diagnosis was verified as well as the smallpox pavilion of the Stockholm Hospital for Infectious Diseases all outcoming material was disinfected in most cases either by incineration or by treatment in a formalin disinfection oven When a patient was removed from an observation ward his room was disinfected with a solution containing 1 % invert soap and 70 % ethanol the floor by washing walls and ceiling by thorough spraying and furniture by washing or spraying A gas mask had to be used during this procedure All textiles and at the beginning also foam rubber mattresses and pillows were treated in a formalin disinfection oven Later on the mattresses were placed in plastic bags which were disinfected simply by wiping off with 5 % chloramine Before the observation wards were taken into use for other patients after the outbreak, they were carefully cleaned and left open to air and sunlight for two days

The same schedule of disinfection and cleaning was used at the Southern Hospital when a smallpox case (E W 3 4) was discovered at a dermatological ward

The outpatient departments at S t Goran's Hospital which were visited by a smallpox case (A A 4 7) on June 6 were disinfected by washing and spraying with chloramine as mentioned above

At the Norrtull Hospital and at the terminal disinfection of the smallpox pavilion of the Stockholm Hospital for Infectious Diseases a combination of washing with chloramine and formalin fumigation was used Thus at the latter place medicaments and much of the equipment was disposed of by incineration and whenever possible other removable equipment was treated in the formalin disinfection oven or, otherwise, washed with 5 % chloramine solution The whole pavilion was then formalin fumigated After a thorough cleaning followed by exposure to air and sunlight for a couple of days it was again taken into use

Discussion and summary of epidemiological features

On April 6 a fairly well vaccinated seaman newly arrived to Sweden from the Far East, developed a mild smallpox for which he did *not* see a doctor He caused an outbreak of smallpox involving 26 further cases

The seaman (*1 1*) was during his illness mainly in contact with persons in his family environment and infected

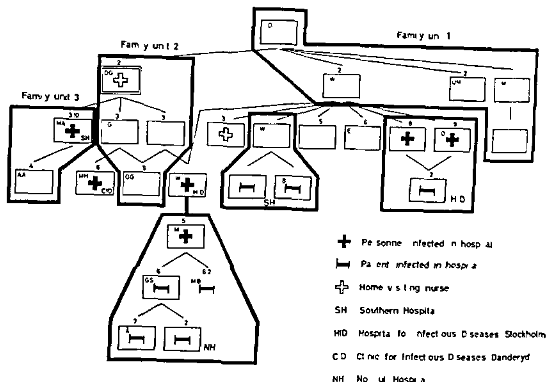


Fig. 8. Epidemiological units.

his grandmother (2.2), his fiancee (2.4) and her sister (2.3), who later infected her fiance (3.7) (Fig. 8, Family unit 1). Outside this family circle the grandmother's nurse (2.1) spread smallpox to her family (Family unit 2), namely her husband (3.1), nephew (3.2) and brother-in-law (4.5). After her death she gave rise to variola in the woman who laid out her corpse (3.10) and this woman infected her mother (4.7) (Family unit 3). Thus the infection in the beginning of the outbreak spread within family circles. As in most European smallpox epidemics in recent years the infection was, however, relatively quickly introduced into hospitals (5). D.G. (2.1) was admitted to the Southern Hospital on April 23, where she gave rise to one secondary case, the layer-out (3.10) (see above), while A.W. (2.2), who was admitted to the Stockholm Hospital for Infectious Diseases on April 27 on account of chicken pox, caused two secondary cases among the hospital personnel (3.8 and 3.9), who later infected a one-year-old girl (4.2), a patient in their ward.

The first recognized case (3.2), his uncle (3.1) and E.W. (3.4) were also cared for at various hospitals in Stockholm for several days before the smallpox diagnosis was established. The case (3.2) infected a nurse (4.6), while 3.1 or 2.2 — possibly through indirect contact, contaminated bedclothes etc. — infected a hospital worker (4.1) at the Stockholm Hospital for Infectious Diseases. E.W. (3.4) during her stay at the Southern Hospital gave rise to two secondary cases among her co-patients (4.3 and 4.4).

1000 m

Imported case

C

B

D

A

Fig 9 Place of infection of smallpox patients in Stockholm 1963
A Southern Hospital
B Stockholm Hospital for Infectious Diseases
C Danderyd Cl nic for Infectious Diseases
D Norrtull Hosp tal

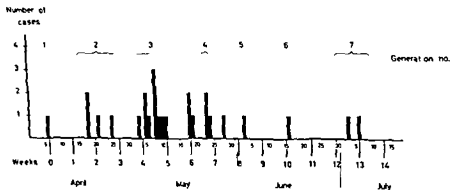


Fig 10 Date of onset ?6 clinical cases.

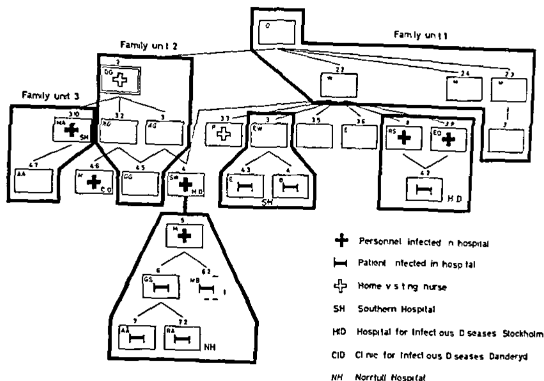


Fig 8 Epidemiological units

his grandmother *(2 2)*, his fiancee *(2 4)* and her sister *(2 3)*, who later infected her fiance *(3 7)* (Fig 8 Family unit 1) *Outside this family* circle the grandmother s nurse *(2 1)* spread smallpox to her family (Family unit 2), namely her husband *(3 1)*, nephew *(3 2)* and brother in law *(4 5)* *After her death she gave rise to* variola in the woman who laid out her corpse *(3 10)* and this woman infected her mother *(4 7)* (Family unit 3) Thus the infection in the beginning of *the outbreak spread within family circles* As in most European smallpox epidemics in recent years the infection was, however, relatively quickly introduced into hospitals (5) *D G (2 1) was admitted to the Southern Hospital on April 23,* where she gave rise to one secondary case, the layer out *(3 10)* (see above), while *A W (2 2) who was admitted to the* Stockholm Hospital for Infectious Diseases on April 27 on account of chicken pox caused two secondary cases among the hospital personnel *(3 8* and *3 9)* who later infected a one year old girl *(4 2)*, a patient in their ward

The first recognized case *3 2)* his *uncle (3 1)* and *E W (3 4)* were also cared for at various hospitals in Stockholm for several days before the smallpox diagnosis was established The case *(3 2)* infected a nurse *4 6* while *3 1* or *2 2* — possibly through indirect contact contaminated bedclothes etc infected a hospital worker *4 1* at the Stockholm Hospital for Infectious Diseases *E W (3 4), during her stay at* the Southern Hospital gave rise to two secondary cases among her co-patients *(4 3* and *4 4)*

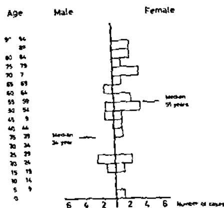


Fig 12 Age and sex distribution of smallpox patients 27 cases

TABLE 5 Summary of vaccinal state of smallpox patients

Vaccinal state	Number of cases	Deaths
Not vaccinated	3	2
Primarily vaccinated	19	2
Revaccinated	5	0
Total	*27*	*4*

vaccinations and isolations and the 60 year-old hospital worker (*4 1*), who carried the infection to Norrtull Hospital The fifth generation comprised only one case which was however not diagnosed until a case had occurred in the sixth generation and persons in the seventh generation had been infected The latter were however isolated before falling ill Thus as appears from Fig 11 and Table 7 page 54—55 6 cases were isolated only after the end of their illness and 14 while they were still ill The remaining 7 cases were isolated before falling ill One patient (*6 2*), who exhibited no clinical symptoms but had a rise of titre at the time of isolation has been referred to the group "ill at time of isolation infectious

Attempts to isolate variola virus were made in 20 of the smallpox cases and were successful in 16

Of the 27 cases 21 were women and 6 men Fig 12) The median age for women was 55 years and for men 34 This sex distribution was undoubtedly due primarily to epidemiological circumstances A W (2 2) for example had contact almost exclusively with women six of whom contracted smallpox Many cases occurred among the predominantly female hospital personnel The infection was also carried to Norrtull Hospital which has only female patients

Vaccinal state of the smallpox cases

The vaccinal state of the 27 smallpox cases is shown in Fig 13 and Table 5 Three had never been vaccinated against smallpox at the time of exposure two of them died Nineteen had only had a primary vaccination all at least 14 years previously and 14 more than 40 years previously two of the latter died Only 5 of the smallpox cases had been revaccinated 3 within the past 3 years the other 2 more than 15 years previously None of them died and those vaccinated within 3 years developed a very mild smallpox two of them without eruptions Thus the majority of the smallpox cases had an unsatisfactory vaccinal state and all the fatal cases could be considered unprotected at the time of exposure

Seven of the smallpox cases were vaccinated during the incubation period

Isolated after recovery or end of infectious period

Ill at time of isolation Infectious

Epidemiological risks

Isolated before onset of illness

Fig 11 Isolation of smallpox patients

The *hospital worker (4 1)* carried the infection to Norrtull Hospital where it spread via a nurse's assistant (*5 1*) to the patients causing two generations of cases two patients in each generation

Altogether 27 smallpox cases occurred As appears from Fig 8 7 inlying patients and 6 staff members were infected in hospitals Furthermore two home visiting nurses were afflicted

Of the remaining 11 cases (the import case not counted) 10 were close family contacts for one (E A *3 6*) the contact was more transient She lived in the same house as A W (*2 2*) and was probably infected by her The two women did not know each other but may have met casually on the stairs or in the lift

As shown on the map (Fig 9) all the smallpox cases contracted their infection in the Greater Stockholm Area

As may be seen from Fig *10* the onset of 26 cases with clinical symptoms of smallpox occurred in waves with roughly two weeks between each wave When the first diagnosis of smallpox was made on May 13 1963 all cases in the first three generations 15 in all had already *fallen ill and all 7 cases in the fourth* generation may have been infected By May 16 after extensive investigations 13 of the cases in the first three generations had been traced and isolated The remaining two cases were nurse's assistant (*3 8*) at the Stockholm Hospital for Infectious Diseases who only had a short period of fever and case *3 10* the layer out whose illness was so mild that the patient did not visit a doctor

In the fourth generation *5 of the 7* cases were isolated before falling ill The other two patients were the 84 year-old woman A A (*4 7*) whose visit to St Goran Hospital necessitated extensive

TABLE 6 Staff of reinforced Epidemiological Department at NBL

	Administration	Home visiting groups	Epidemiological field groups	Vaccination groups	Information section	Registration section	*Total*
Physicians							
Epidemiologists	1		3			1	*5*
Bacteriologists				2	1		*3*
Virologists	1						*1*
Clinicians		3			2		*5*
Public Health Inspectors	1	1	2	4	1	3	*12*
Nurses							
Qualified nurses with epidemiological experience	1				3		*4*
Red cross auxiliary nurses with epidemiological experience						2	*2*
Red cross auxiliary nurses						4	*4*
Secretaries	3				2	1	*6*
Total	*7*	*4*	*5*	*6*	*9*	*11*	*42*

For two of them this was their primary vaccination given about 6 days before the onset of the disease One case was revaccinated probably on the day before contact H M 5 1) another within 24 hours after contact (M H 4 6) Both contracted smallpox but had comparatively mild symptoms

Organization and staffing of the epidemiological work

The supervision of public health measures in Sweden lies with the National Board of Health This authority maintains contact with WHO and issues reports and instructions, e g during serious epidemics Far reaching decisions such as that of mass vaccination are taken at this level The epidemic situation in the country is continuously followed by the Epidemiological Department of the National Bacteriological Laboratory (NBL) which takes action when considered necessary However the primary responsibility for the epidemiologic field work lies with the regional and local public health boards At a meeting of the National Board of Health on May 14 one group of specialists was appointed to deal with the hospital care

Serial number	Time before onset				Incubation period	Onset	After onset	
	Years	Months	Weeks	Days	Days		Days	Weeks
1 1								
2 1 †								
2 2								
2 3								
2 4								
3 1								
3 2 †	Not vaccinated							
3 3								
3 4								
3 5								
3 6								
3 7								
3 8								
3 9								
3 10								
4 1								
4 2	Not vaccinated before exposure							
4 3 †								
4 4								
4 5 †	Not vaccinated before exposure				1)			
4 6								
4 7								
5 1				2)				
6 1							(O)23 6 -63	
6 2								
7 1								
7 2								

■ Successful primary vaccination
⊠ ? successful primary vaccination
● Successful revaccination
⊙ ? successful revaccination
○ Unsuccessful revaccination

1) Within one day after exposure
2) Day before exposure

Fig 13 Vaccinal state of smallpox patients

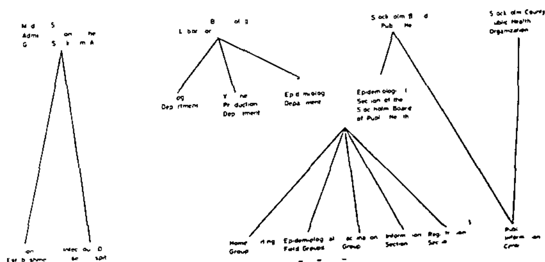


Fig 14 Organization chart

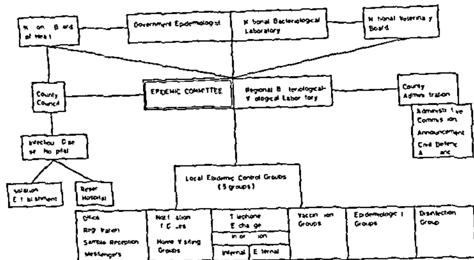


Fig 15 Organization plan for the combating of epidemics in a county

public health inspectors These groups took turns of daily duty and were required to arrange at short notice extensive vaccinations of persons in the environment of suspect and known cases of smallpox

An Information Section was organized with the main purpose of keeping doctors and public health authorities informed of the situation within the epidemic area and in the country as a whole

In the initial stage, however the Information Section soon became inundated with inquiries from the general public The telephone switchboards to the NBL and other bacteriological laboratories as well as to the National Board of Health hospital boards and infectious disease hospitals were blocked

A separate *Public Information Centre* was therefore established with a 38 line switchboard and some 20 personnel from the Women s Voluntary Service under the leadership of doctors one doctor per five personnel The centre answered questions of a simple nature from the public and passed on inquiries of significance for control of the epidemic to the proper authorities and to the Epidemiological Department

During the first three weeks of the epidemiological work representatives of public health authorities and hospitals met at the NBL every evening under the chairmanship of the Government Epidemiologist to study and discuss the situation and issue the necessary directives

Epidemic Committee

In conjunction with the smallpox outbreak in the Greater Stockholm area in 1963 there were fairly satisfactory facilities for forming ' combat groups of epidemiologically well trained specialists representing many branches of medicine In the provinces on the other hand, the

and another with the epidemiological field work. The head of the Public Health Office of the National Board of Health was made responsible for contact with mass media on administrative questions, the head of the Stockholm Hospital for Infectious Diseases on clinical questions, and the Government Epidemiologist (head of the Epidemiological Department at the NBL), on epidemiological questions.

The Organization Chart (Fig. 14) shows the various authorities in charge of the 1963 outbreak in Stockholm.

The chief responsibility of the Medical Services Administration of the Greater Stockholm Area was the organization of isolation establishments for smallpox contacts.

Two departments of the NBL, in addition to the Epidemiological one — the Virological and the Vaccine Production Departments — were actively engaged in combating the epidemic.

The main function of the *Virological Department* was the laboratory examination of specimens from a very large number of cases suspected of smallpox.

The *Vaccine Production Department* was responsible for the control, dispensing and distribution of smallpox vaccine throughout the country.

The *Epidemiological Department*, aided by specially recruited doctors and nurses and combined with the *Epidemiological Section of the Stockholm Board of Public Health* (Table 6) had responsibility for control of the outbreak. It also acted as advisory body to hospitals, physicians and authorities, both within the epidemic area and in the country as a whole, on questions concerning control measures.

At the beginning of the outbreak practitioners referred a great many cases of exanthemateous disease to the infectious disease hospitals on the suspicion of smallpox. This placed an extreme strain on these hospitals. To prevent chaos, three physicians were asigned the task of visiting such cases in their homes. Two of these doctors were infectious disease specialists, another had studied smallpox abroad. Many cases of exanthemateous disease were eliminated in this way. In some cases patients were isolated in their homes pending virological examination.

An essential function in the control of the smallpox outbreak was fulfilled by Epidemiological Field Groups, consisting of an epidemiologist, assisted by nurses or public health inspectors. These groups carried out investigations in the field, tracing contacts of smallpox cases, suspect cases, etc. Action, such as isolation and vaccination could often be ordered immediately. Close contact was maintained with the infectious disease hospitals and the isolation establishments in order to procure anamnestic data of importance for the organization of preventive measures. This was done at an early stage of the illness, even before a diagnosis could be made on the basis of virological tests.

In certain serious situations extremely great demands were made on the field groups, for instance after the visit of a woman with smallpox, V. A. (4.7), to St Goran Hospital. All epidemiological data were card indexed and analysed in the Registration Section.

Vaccination Groups were organized consisting of a physician assisted by

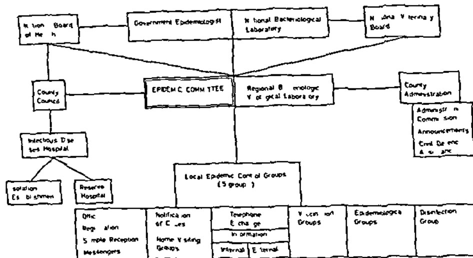


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APPENDIX

Apparatus for Mass Vaccination Against Smallpox

NILS PETERSON[1]

An electrically operated apparatus for smallpox vaccination has been designed in order to eliminate the usual lengthy manual procedure of the multiple pressure method (Fig 16)

A vibrator is fitted with a platinum needle which can be very quickly sterilized by electrical heating In preliminary trials of primary vaccination on infants, practically 100 % takes were obtained with the apparatus when a vaccin containing about $10^{7.4}$ TCID$_{50}$ (monkey kidney) per ml was used (3 4)

Before the smallpox outbreak in Stockholm in 1963 the apparatus had been tried only for primary vaccinations It was manufactured in large numbers in the event of mass vaccination being

[1] Present adress Central Bacteriological Laboratory of Stockholm City Box 177 Stockholm 1

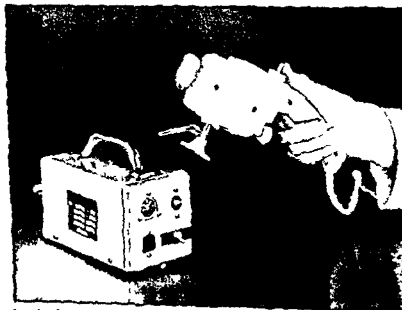


Fig 16 Vaccination apparatus with platinum needle and transformer

personnel resources are more limited The spread of the outbreak to a provincial area would have resulted in a weakening of the personnel resources in the Greater Stockholm region — the detachment of epidemiologically well-trained personnel from Stockholm to the county in question would in such case have been inevitable The responsible authorities have therefore considered it necessary to improve the provincial resources Therefore the National Board of Health has requested the county authorities to form epidemiological committees for planning and supervising the fighting of major epidemics

These committees would consist of

1 Senior County Medical Officer
2 Assistant County Medical Officer
3 County Sanitary Adviser
4 Representative of County Council
5 Representative of County Administrative Board
6 Head of County Hospital for Infectious Diseases
7 County Veterinary Officer
8 County Engineer (Water and Sewage)

The job of the committee would be to plan for the combating of epidemics such as smallpox, salmonella and infectious hepatitis

The committee should arrange for emergency supplies of certain materials (chiefly for vaccination and sampling) and for accommodation of the individual units, which may be organized as shown in Fig 17 It should also be responsible for the recruitment of personnel for the individual units by concluding an agreement with the County Council, under which specially trained doctors and nurses would be made available in the event of a major or serious epidemic By these means the resources of the county would be better utilized and countermeasures could be taken at an earlier stage than has hitherto been possible

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4 Espmark Åke Standardisering av smittkoppsvacciners styrka Thesis Stockholm 1964

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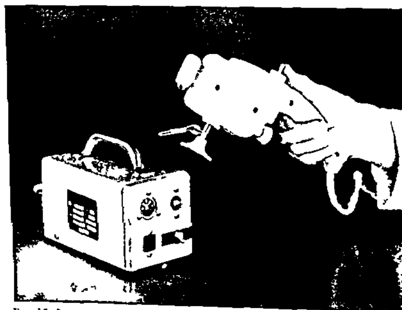


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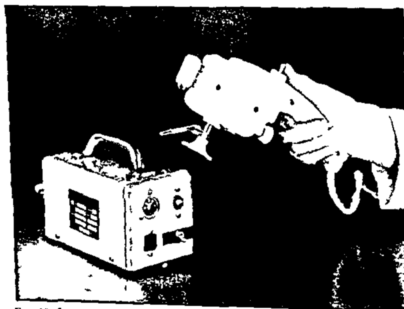


Fig 16 Vaccination apparatus with platinum needle and transformer



Fig 17 Apparatus equipped with special device for application of vaccin to the skin

needed on epidemiological grounds Al together some 50,000 persons were vaccinated with this apparatus The vaccinator could vaccinate up to 500 persons per hour, provided that he had 4—5 assistants a physician inquiring about medical contraindications, and assistants for recording and guiding of vaccinees, wiping the skin and applying the vaccine Takes were obtained in 97 % To *achieve this degree of success the vac* cinator must be experienced in use of the apparatus, but this experience can be quickly acquired

The apparatus can operate off a storage battery, which permits its use also in the field

A further development includes automatic dispensing of the vaccine dropwise (Fig 17)

The area to be treated is the same as in the multiple pressure method i e about 3 mm diameter

Patents for the apparatus have been filed in six countries It is made by AB Relax, Odengatan 39, Stockholm

CHAPTER II

Case Histories

(as summarized by authors of chapters I and III)

This chapter contains a brief descrip tion of each of the 27 smallpox cases with essential epidemiological and clin ical data Some of these are given in Table 7 and Fig 1 In the numbering of the cases the first digit indicates the generation the second the order in which cases were recognized within each generation

Case 1 1 L O

L.O was a 23 year-old seaman who since the autumn of 1962 had worked as a mechanic on a Swedish tanker on an Indonesian coastal trade On February 28 1963 he signed off at Derby Australia On March 22 he left Perth by air on the return journey to Stock holm with intermediate landings at Jakarta Singapore Rangoon Calcutta Karachi Teheran Damascus Zurich Dusseldorf and Copenhagen Apart from a day s stay in Zurich he never left the transit areas

After his return to Stockholm on March 24 1963 he lived mostly with his grand mother Mrs A W (2 2) but temporarily also with his fiancée Miss U M (2 4) who sometimes visited A W He also visited his parents who lived some 40 miles from Stockholm both before falling ill and one week after The latter however were un affected

On his arrival in Stockholm the seaman was fully healthy but on April 6 fell ill with fever On the same or on one of the following days eruptions developed on the face and back of the neck and on the arms and legs He was febrile for three days and thereafter recovered completely without complications

As L.O had often been ill on return from his travels and had earlier suffered from acne he paid no particular attention to his symp toms He did not visit a doctor or hospital During the acute stage he remained for the most part quietly at home (for about one week) During his illness he was cared for by his grandmother A W (2 2) but was also in contact with the nurse D G (*2 1*) who daily visited his grandmother and with his fiancee U M (*2 4*) After his recovery he returned to work as mechanic at a lamp factory in Stockholm

L.O had been successfully vaccinated against smallpox as a child and again in 1955 1956 and 1959 (successfully on one occasion) and during military service in 1960 (successfully) His last vaccination be fore his illness had been on May 22 1961 at Port Said (successful according to his own report)

The diagnosis of variola was based on the following conditions

1 Epidemiological data
2 Clinical course
3 Blood samples collected five weeks after the onset showed high titres in H I and C F tests although the patient had not been vac cinated during the last two years
4 Vaccination on May 16 1963 with extra potent vaccine was unsuccessful

Dixon s type 7 mild variola (1)

Case 2 1 D G

Mrs D G 57 years wife of A G (*3 1*) and aunt of K G (*3 2*) She worked in different homes as a home visiting nurse for old people From March 26 to April 11 she worked daily at the home of A W (*2 2*)

where L O (1 1) was ill from April 6 to about April 11

D G had been vaccinated against smallpox in 1920 She fell ill on April 18 with high fever, headache and muscular pain She was treated at home with antibiotics but remained febrile On April 22 widespread petechial cutaneous haemorrhages appeared, and on the following day violent haemorrhagic manifestations (haematuria, melaena) She was admitted to the Southern Hospital in Stockholm on the evening of April 23 in poor condition and died about 2 hours later in a picture of shock No exanthema was observed Blood tests showed only 24 000 thrombocytes

Postmortem (A Moberg M D)
The most impressive autopsy finding was an immense number of cutaneous petechiae evenly distributed over the entire body without any sign of vesiculation There were also intramural myocardial haemorrhages (also involving the pars membranosus), and haemorrhages in the gastric mucosa, the renal pelves and the urinary bladder There was also a heavy pulmonary oedema Other organs had only slight changes compatible with the age of the patient

The major microscopical observation was severe bone marrow aplasia involving all components and for which no cause could be given at the time No section were taken from the skin A diagnosis of aplastic anaemia with marked thrombocytopenia was suggested

Since D G died before it was known that smallpox existed in Sweden the etiology diagnosis of her illness became evident only when epidemiological data were also available

Dixon s type 1 Purpura variolosa

During her illness at home D G infected her husband A G (3 1) and his nephew K G (3 2) The infection was also transmitted to Miss M A (3 10) who laid out the corpse of D G

Case 2 2 A W

Mrs A W 80 years of age was grandmother of the seaman L O (1 1) who had lived with her after his return from Australia on March 24, 1963

A W had been vaccinated against smallpox as a child but had not been revaccinated and denied having had chickenpox She nursed her grandson during his illness and fell ill herself with fever around April 21 On April 23 an exanthema appeared starting on the arms and later spreading to the face and trunk She was admitted to the Stockholm Hospital for Infectious Diseases on April 27 with the diagnosis of chickenpox

On admission she was afebrile and completely unaffected, the exanthema being rather sparse and consisting of small vesiculopapules, some eruptions were however papulous in the early stage and some scabby There were eruptions also in the folds of the skin The illness was taken to be chickenpox and the patient was sent home on May 7 She was afebrile throughout her time in hospital After recognition of the smallpox outbreak she was readmitted on May 15, the efflorescences were then scabby on the body, palms soles and scalp

The diagnosis of smallpox was verified serologically

Dixon's type 6 discrete variola

During her illness A W was nursed at home by her grandson L O (1 1) and her daughter in law Mrs E W (3 4) and by a number of visiting nurses and family friends

During her early period at the Stockholm Hospital for Infectious Diseases A W was isolated in a single room at an observation ward without any rigid precautions being taken Here she gave rise to two secondary cases among the nursing staff (3 8 and 3 9)

Case 2 3 M M

Miss M M a 23-year-old clerk was engaged to K C (3 7) and sister of U M (2 4) She was infected by L O (1 1) who was engaged to U M During his illness L O had visited the two sisters in their home

M M who had been vaccinated against smallpox successfully in 1943 had not had chickenpox She fell ill on April 25 with high fever muscular pain and headache Four

days later a rash on arms and legs on the face and scalp appeared The rash developed in due course into vesicles pustules and scabs She was examined by a doctor on April 27 and 30 and thought to have chicken pox She returned to work on May 13 Two days later she was admitted to the Hospital for Infectious Diseases for examination and then had scabs on the extremities face and scalp Variola virus was isolated from scab samples collected on May 15

Dixon s type 6 discrete variola

During the entire acute phase of her illness M M was nursed at home by her sister U M (2 4) and fiancé K C (3 7) and for a few days by her mother who however escaped infection

Case 2 4 U M

Miss U M, 19 years of age was a clerk like her sister M M (2 3) She was in daily contact with her fiancé L O (1 1) after his return on March 24 and at times nursed him during his illness (April 6—11)

U M who had not had chickenpox had been vaccinated successfully against smallpox around 1950 One evening around April 16 she developed fever and on the following days headache dizziness and pain in the lumbar region She stated that she had a few small eruptions on the chin She rapidly recovered and had no scars From clinical and serological data she was classified as a case of

Dixon s type 8 abortive variola

Case 3 1 A G

A G was a 52 year-old former bricklayer He was married to D G (2 1) and nursed her during her illness from April 18 to 23 before her death of what proved to be haemorrhagic variola He had been successfully vaccinated against smallpox as a child probably in 1915 and had not had chicken pox

A G fell ill on May 3 with fever headache and pain in the back He was seen at the Southern Hospital on the same day but sent home Exanthema appeared on May 6 On May 7 he again presented at the same hospital and was now transferred to the Stockholm Hospital for Infectious Diseases on the account of fever and exanthema of unknown etiology

The exanthema developed into a papulovesicular eruption on the face (Fig 18) scalp extremities and trunk. The efflorescences exhibited different stages of development, appearing also in the folds of the skin No umbilicated pustules appeared His temperature was around 38 5 C at the onset of exanthema gradually falling to afebrility in the course of 12 days An X ray taken three days after admission showed a slight opacity of the left lung

The diagnosis was established serologically and by isolation of virus

Dixon s type 6 discrete variola

During his illness at home A G was nursed by his brother O G (4 5) and the latter s wife F G who had come to Stockholm to attend the funeral of D G (2 1) Apart from the three latter three other persons were present at the funeral on May 6 but with the exception of O G (4 5) none of them fell ill

Case 3 2 K G The first recognized case

K G was a 19 year old bricklayer He lived mostly with his fiancée but visited his aunt D G (2 2) and uncle A G (3 1) several times a week He had visited them on April 19 the day after D G had developed small pox

K G had had chickenpox as a child He had not been vaccinated against smallpox He fell ill on May 5 with high fever headache vomiting and pain in the lumbar region An exanthema appeared two days later first on the hands then spreading to the rest of the body On May 8 he was admitted to the Clinic for Infectious Diseases of the Danderyd Hospital He was then highly febrile and had a general exanthema consisting of small bright red maculopapules predominantly on the backs of the hands somewhat less on the neck and chin trunk and feet and only a few on the legs On admission he was believed to suffer from sepsis with trombocytopenia He later developed a severe membraneous stomatitis purulent conjunctivitis and sores

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The major microscopical observation was severe bone marrow aplasia involving all components and for which no cause could be given at the time No section were taken from the skin A diagnosis of aplastic anaemia with marked thrombocytopenia was suggested

Since D G died before it was known that smallpox existed in Sweden the etiology diagnosis of her illness became evident only when epidemiological data were also available

Dixon s type 1 *Purpura variolosa*

During her illness at home D G infected her husband A G (3 1) and his nephew K G (3 2) The infection was also transmitted to Miss M A (3 10) who laid out the corpse of D G

Case 2 2 A W
Mrs A W 80 years of age, was grandmother of the seaman L O (1 1) who had lived with her after his return from Australia on March 24, 1963

A W had been vaccinated against smallpox as a child but had not been revaccinated and denied having had chickenpox She nursed her grandson during his illness and fell ill herself with fever around April 21 On April 23 an exanthema appeared starting on the arms and later spreading to the face and trunk She was admitted to the Stockholm Hospital for Infectious Diseases on April 27 with the diagnosis of chickenpox

On admission she was afebrile and completely unaffected, the exanthema being rather sparse and consisting of small vesiculopapules, some eruptions were however, papulous in the early stage, and some scabby There were eruptions also in the folds of the skin The illness was taken to be chickenpox and the patient was sent home on May 7 She was afebrile throughout her time in hospital After recognition of the smallpox outbreak she was readmitted on May 15, the efflorescences were then scabby on the body, palms soles and scalp

The diagnosis of smallpox was verified serologically

Dixon s type 6 discrete variola

During her illness A W was nursed at home by her grandson L O (1 1) and her daughter-in law Mrs E W (3 4) and by a number of visiting nurses and family friends

During her early period at the Stockholm Hospital for Infectious Diseases A W was isolated in a single room at an observation ward without any rigid precautions being taken Here she gave rise to two secondary cases among the nursing staff (3 8 and 3 9)

Case 2 3 M M
Miss M M a 23 year old clerk was engaged to K C (3 7) and sister of U M (2 4) She was infected by L O (1 1) who was engaged to U M During his illness L O had visited the two sisters in their home

M M who had been vaccinated against smallpox successfully in 1943 had not had chickenpox She fell ill on April 25 with high fever, muscular pain and headache Four

days later a rash on arms and legs on the face and scalp appeared The rash developed in due course into vesicle pustules and scabs She was examined by a doctor on April 27 and 30 and thought to have chicken pox She returned to work on May 13 Two days later she was admitted to the Hospital for Infectious Diseases for examination and then had scabs on the extremities face and scalp Variola virus was isolated from scab samples collected on May 15

Dixon s type 6 discrete variola

During the entire acute phase of her illness M M was nursed at home by her sister U M (2 4) and fiancé K C (3 7) and for a few days by her mother who however escaped infection

Case 2 4 U M

Miss U M 19 years of age was a clerk like her sister M M (2 3) She was in daily contact with her fiancé L O (1 1) after his return on March 24 and at times nursed him during his illness (April 6—11)

U M who had not had chickenpox had been vaccinated successfully against smallpox around 1950 One evening around April 16 she developed fever and on the following days headache dizziness and pain in the lumbar region She stated that she had a few small eruptions on the chin She rapidly recovered and had no scars From clinical and serological data she was classified as a case of

Dixon s type 8 abortive variola

Case 3 1 A G

A G was a 52 year-old former bricklayer He was married to D G (2 1) and nursed her during her illness from April 18 to 23 before her death of what proved to be haemorrhagic variola He had been successfully vaccinated against smallpox as a child probably in 1915 and had not had chicken pox

A G fell ill on May 3 with fever headache and pain in the back He was seen at the Southern Hospital on the same day but sent home Exanthema appeared on May 6 On May 7 he again presented at the same hospital and was now transferred to the Stockholm Hospital for Infectious Diseases on the account of fever and exanthema of unknown etiology

The exanthema developed into a papulovesicular eruption on the face (Fig 18), scalp extremities and trunk The efflorescences exhibited different stages of development appearing also in the folds of the skin No umbilicated pustules appeared His temperature was around 38 5 C at the onset of exanthema gradually falling to afebrility in the course of 12 days An X ray taken three days after admission showed a slight opacity of the left lung

The diagnosis was established serologically and by isolation of virus

Dixon s type 6 discrete variola

During his illness at home A G was nursed by his brother O G (4 5) and the latter s wife E G who had come to Stockholm to attend the funeral of D G (2 1) Apart from the three latter three other persons were present at the funeral on May 6 but with the exception of O G (4 5) none of them fell ill

Case 3 2 K G The first recognized case

K G was a 19 year old bricklayer He lived mostly with his fiancée but visited his aunt D G (2 2) and uncle A G (3 1) several times a week He had visited them on April 19 the day after D G had developed small pox

K G had had chickenpox as a child He had not been vaccinated against smallpox He fell ill on May 5 with high fever headache vomiting and pain in the lumbar region An exanthema appeared two days later first on the hands then spreading to the rest of the body On May 8 he was admitted to the Clinic for Infectious Diseases of the Danderyd Hospital He was then highly febrile and had a general exanthema consisting of small bright red maculopapules predominantly on the backs of the hands somewhat less on the neck and chin trunk and feet and only a few on the legs On admission he was believed to suffer from sepsis with trombocytopenia He later developed a severe membraneous stomatitis purulent conjunctivitis and sores

on the glans penis and around anus The picture then was similar to that of a mucocutaneous syndrome After some days, however, vesiculopustules started to develop especially on the face and arms (Fig 19) The condition was diagnosed as smallpox on May 13 through virological analyses of efflorescence samples and serum He was transferred to the Stockholm Hospital for Infectious Diseases The exanthema developed rapidly and generally into umbilicated partially confluent pustrules on a haemorrhagic base (Fig 20) There was at first a slow improvement, thereafter increasing deterioration with impairment of various organs resulting in signs like rise of nonprotein nitrogen, elevated transaminase and, towards the end, fairly strong signs of cerebral affection, terminating in circulatory collapse and death on May 28 He had been treated with methisazone (Marboran) vaccinia immune globulin, antibiotics, shock therapy including blood transfusions and, at an early stage of the disease, steroids

Dixon s type 4 benign confluent variola

During his time at the Clinic for Infectious Diseases of the Danderyd Hospital he was in contact mainly with the nursing staff He gave rise to one secondary case, Mrs M H (*4 6*), who nursed him during the night between May 13 and 14 During his illness he had been in contact also with his parents and his fiancée The latter had been successfully vaccinated in 1962

Case 3 3 K P

Mrs K P was a 66 year old woman who, like D G (*2 1*) worked as a home visiting nurse for old people On April 24 she nursed A W (*2 2*) who had developed smallpox three days previously

K P had had chickenpox She had been vaccinated against smallpox as a child and again in 1918 She fell ill quite suddenly on May 8 with shivering headache and 39 C fever which successively fell to afebrility in the course of 5 days She was thereafter essentially free from symptoms and had no exanthema Revaccination was unsuccessful

Her diagnosis was confirmed serologically

Dixon s type 9 variola sine eruptione

During her illness K P remained quietly at home She was admitted to the Stockholm Hospital for Infectious Diseases on May 14 for examination due to her contact with A W

Case 3 4, E W

Mrs E W , 55 years of age, was daughter in law of A W (*2 2*) and visited her during her illness both at home on April 23 and 26 and at the Stockholm Hospital for Infectious Diseases on April 28 and May 5

E W had been successfully vaccinated against smallpox as a child She had not had chickenpox

She fell acutely ill on May 6 with headache, pain in the lumbar region and high fever around 40 C

On May 9 she was admitted to a medical ward of the Southern Hospital on the suspicion of pyelitis Her temperature fell almost critically on May 10 when a papulous exanthema appeared especially on the face and arms accompanied by acute smarting pains

The exanthema spread quickly to the trunk extremities, palms, soles and scalp and gradually developed into vesicles pustules and scabs The exanthema was initially thought to be of allergic origin and due to sulpha treatment Steroids were therefore given and on May 14 the patient was transferred to the dermatological clinic of the same hospital On May 15 the efflorescences on the face were variola like while those on the trunk resembled varicellae (Fig 21) Vesicles were also observed in the mouth and pharynx In scrapings from blisters on May 15 the cell cytoplasm was found to contain *acidophilic inclusion bodies* The same day she was admitted to the Stockholm Hospital for Infectious Diseases.

The diagnosis of smallpox was verified serologically and by isolation of variola virus

Dixon s type 6 discrete variola

Two elderly women (*4 3* and *4 4*) who had been room mates of E W in the medical ward developed smallpox

Case 3 5 A F

Mrs A F was a 50-year-old woman who had earlier had chickenpox She was successfully vaccinated against smallpox in 1918 On April 26 A F visited A W (*2 2*) who then had a pronounced exanthema A F fell acutely ill on May 5 with headache pains in muscles and joints shivering and high fever On the sixth day an erythema devel oped on the face and over the chest An exanthema developed on the same day with uniform maculopapules appearing simulta neously on the face scalp trunk and extrem ities The eruption became vesicular pustulous and incrustated in the ordinary way The efflorescences were small in size (Fig 22) She had a moderate secondary fever lasting nine days

The diagnosis was verified serologically and by isolation of virus

Dixon s type 6 discrete variola

After falling ill on May 5 A F was nursed at home until May 14 when she was admitted to the Stockholm Hospital for Infectious Diseases At home she was cared for by her husband and three children No member of the family had a satisfactory vaccinal state (one son had never been vaccinated) yet none of them developed smallpox

Case 3 6 E A

Mrs E A was a 71 year-old woman who had had chickenpox She was successfully vac cinated as a child around 1900 and revaccina ted in 1915 She lived in the same apartment house as A W (*2 2*) who had been ill with smallpox at home from April 21—27 Al though the two women were unaquainted they might have met on the stairs or in the lift Alternatively E A who is not known to have had contact with any other case of smallpox might have been infected more indirectly possibly via the ventilation system A W lived on the first floor E A on the third No other case occurred in that house

She fell ill on May 8 with shivering and fever backache and headache Three days later a profuse but non-confluent exanthema developed on the arms and later spread to the face scalp legs and trunk also to the palms and soles A doctor who visited the patient at home on May 13 diagnosed her condition as varicellae On May 16 however, she was hospitalized as a suspect case of smallpox At the beginning of the exanthem atous stage her temperature was around 38 5 C, gradually falling to afebrility after some 3 weeks (Fig 23) No complications developed

The diagnosis was verified serologically and by isolation of virus

Dixon s type 6 discrete variola

E.A caused no secondary cases

Case 3 7 K C

K C. 21 years of age was fiancé of M M (*2 3*) who developed smallpox on April 25 and was thereafter nursed at home He lived for most of the month of April with his fiancée and nursed her during her illness Before falling ill K C. was also in contact with his fiancée s sister L M (*2 4*) and the seaman L.O (*1 1*)

K C had been successfully vaccinated as a child and again on military service in 1961 He fell ill on May 11 with fever 38 4 C, headache and pains in the right side of the chest On May 15 he was isolated at the Stock holm Hospital for Infectious Diseases By then he was afebrile and free from symptoms Revaccination with extra potent vaccine produced no reaction The case was verified serologically

Dixon s type 9 variola sine eruptione

Case 3 8 R S

Miss R S was a 44 year-old nurse s assistant who worked in one of the observation wards of the Stockholm Hospital for Infectious Dis eases At times she was in charge of A W (*2 2*) who from April 27 to May 7 was a patient in the ward with the diagnosis of varicellae

R S was vaccinated as a child and there after on several occasions The last vaccina tion reported as successful was in 1962 She fell acutely ill on May 9 with chills fever up to 40—41 C headache muscular pains vomiting and on the third day a rapidly

transient erythema on the body Successive fall of temperature to afebrility without appearance of exanthema Revaccination with extra potent vaccine without take

The diagnosis was verified serologically (Significant fall in antibody titres)

Dixon s type 9 variola sine eruptione

After falling ill R S continued at work from May 9—12 The following two days she stayed at home On May 15 she returned to work but was isolated on May 18 in the Hospital

One of the patients in this observation ward, I L (4 2), later developed smallpox She was probably infected by R S or E O (3 9), both of whom were working in the ward during the first days of their illness

Case 3 9, E O

Miss E O was a 22 year old nurse s assistant who was working in the same observation ward as R S (3 8) while A W (2 2) was nursed there She was successfully vaccinated against smallpox in 1950 and had had chickenpox as a child

She fell ill on May 10, i e 14 days after the admission of A W, with moderate fever and pain in the lumbar region Two days later she had a headache and general muscular tenderness On May 13 she was afebrile and virtually free from symptoms but noticed a few small blisters on the chin, the outside of the upper arms and the wrists On admittance to the hospital on May 15 she had a few papules and vesicles of pinhead size scattered also on the chest the back and in the face The exanthema rapidly receded Repeated revaccinations with extra potent vaccine were unsuccessful

The diagnosis of smallpox was supported by the results of the serological tests

Dixon s type 8 abortive variola

After falling ill on May 10 E O continued at work for two days She stayed at home from May 12—15 when she was isolated in the Hospital for Infectious Diseases

While on duty E O like R S (3 8) may have infected I L (4 2) who was a patient at her observation ward

Case 3 10, M A

Miss M A was a 53 year-old layer-out employed by a firm of undertakers in Stockholm On April 26 she laid out the corpse of D G (2 1) who had died at the Southern Hospital on April 23

M A had been successfully vaccinated as a child and had had chickenpox She fell ill on May 8, feeling unwell and feverish and had muscular pains for a few hours During the following days she noticed a few small eruptions on the nose and in the left groin, but felt otherwise well Her case was missed in the epidemiological work and her symptoms were so mild that she did not consult a physician Thus her illness did not become known until June 6 when her mother A A (4 7) proved to have smallpox At that time M A had small dried residues of efflorescences sparsely distributed over the greater part of the body including the hard palate Revaccination with extra potent vaccine was unsuccessful The diagnosis of smallpox was verified serologically

Dixon s type 7 mild variola

Case 4 1, S W

S W was a 60 year-old hospital worker at the Stockholm Hospital for Infectious Diseases He had been taken on about one month before falling ill He was successfully vaccinated against smallpox in 1949

As far as is known S W s work did not bring him into direct contact with any smallpox case One of his duties however was to collect laundry and refuse from the observation ward in which A W (2 2) was present from April 27 to May 7 and A G (3 1) from May 7 onwards He was probably infected indirectly when handling contaminated laundry or refuse bags

On May 18 he felt slightly unwell and was subfebrile for one day without distinct symptoms Two days later he noticed blisters in the scalp and nape On May 21 he therefore saw a doctor at the hospital and was isolated He then had a moderate number of small papulovesicles distributed over most of the body The transition to pustules and scabs was quick (Fig 24)

transient erythema on the body Successive fall of temperature to afebrility without appearance of exanthema Revaccination with extra potent vaccine without take

The diagnosis was verified serologically (Significant fall in antibody titres)

Dixon's type 9 variola sine eruptione

After falling ill R S continued at work from May 9—12 The following two days she stayed at home On May 15 she returned to work but was isolated on May 18 in the Hospital

One of the patients in this observation ward, I L (*4 2*), later developed smallpox She was probably infected by R S or E O (*3 9*), both of whom were working in the ward during the first days of their illness

Case 3 9, E O

Miss E O was a 22 year-old nurse's assistant who was working in the same observation ward as R S (*3 8*) while A W (*2 2*) was nursed there She was successfully vaccinated against smallpox in 1950 and had had chickenpox as a child

She fell ill on May 10 i e 14 days after the admission of A W with moderate fever and pain in the lumbar region Two days later she had a headache and general muscular tenderness On May 13 she was afebrile and virtually free from symptoms but noticed a few small blisters on the chin the outside of the upper arms and the wrists On admittance to the hospital on May 15 she had a few papules and vesicles of pinhead size scattered also on the chest the back and in the face The exanthema rapidly receded Repeated revaccinations with extra potent vaccine were unsuccessful

The diagnosis of smallpox was supported by the results of the serological tests

Dixon s type 8 abortive variola

After falling ill on May 10 E O continued at work for two days She stayed at home from May 12—15 when she was isolated in the Hospital for Infectious Diseases

While on duty E O like R S (*3 8*) may have infected I L (*4 2*) who was a patient *at her observation ward*

Case 3 10, M A

Miss M A was a 53 year old layer-out employed by a firm of undertakers in Stockholm On April 26 she laid out the corpse of D G (*2 1*) who had died at the Southern Hospital on April 23

M A had been successfully vaccinated as a child and had had chickenpox She fell ill on May 8, feeling unwell and feverish and had muscular pains for a few hours During the following days she noticed a few small eruptions on the nose and in the left groin but felt otherwise well Her case was missed in the epidemiological work and her symptoms were so mild that she did not consult a physician Thus her illness did not become known until June 6 when her mother A M (*4 7*) proved to have smallpox At that time M A had small dried residues of efflorescences sparsely distributed over the greater part of the body including the hard palate Revaccination with extra potent vaccine was unsuccessful The diagnosis of smallpox was verified serologically

Dixon s type 7 mild variola

Case 4 1 S W

S W was a 60 year-old hospital worker at the Stockholm Hospital for Infectious Diseases He had been taken on about one month before falling ill He was successfully vaccinated against smallpox in 1949

As far as is known S W s work did not bring him into direct contact with any smallpox case One of his duties however was to collect laundry and refuse from the observation ward in which A W (*2 2* was present from April 27 to May 7 and A G (*3 1*) from May 7 onwards He was probably infected indirectly when handling contaminated laundry or refuse bags

On May 18 he felt slightly unwell and was subfebrile for one day without distinct symptoms Two days later he noticed blisters in the scalp and nape On May 21 he therefore saw a doctor at the hospital and was isolated He then had a moderate number of small papulovesicles distributed over most of the body The transition to pustules and scabs was quick (Fig 24)

The diagnosis was verified serologically and by virus isolation

Dixon's type 7 mild variola

During the first days of his illness May 18—20 S W continued at work He transmitted the infection to the Norrtull Hospital, where H M (5 1) developed smallpox at the beginning of June

Case 4 2 I L

I L was an 18-month-old girl who was a patient in one of the observation wards at the Stockholm Hospital for Infectious Diseases having been admitted on May 9 in a dehydrated condition with whooping cough and bronchopneumonia She was not previously vaccinated against smallpox She was probably infected by one of the nurses R S (3 8) or E O (3 9) She was vaccinated on May 15 and at the same time given 5 ml of vaccinia immune globulin On May 19 having been afebrile for a week, her temperature suddenly rose to 39 C On the following day she had convulsions when her temperature was at its peak of 40 C, On May 21 small papules some of them vesiculated were observed on the trunk extremities and scalp Her development was typical of discrete variola (Fig 25) She was afebrile after a week and her general condition quickly improved No complications occurred On the fifth to eighth days after the outbreak of exanthema she received 0 5 g methisazone twice daily She was discharged in good condition but had pockmarks especially on the forehead Two years afterwards however these are just barely visible

The diagnosis was verified serologically and by isolation of virus

Dixon's type 6 discrete variola

Case 4 3 E T

Mrs E T was a 72 year-old woman who had been admitted to the Southern Hospital for cardiac insufficiency During May 9—14 she shared a room with E W (3 4) who was in the hospital on the suspicion of pyelitis E T who had been vaccinated as a child was revaccinated on May 16 (successfully) She was isolated on May 15 and three days later transferred to the Clinic for Infectious Diseases of the Danderyd Hospital She fell acutely ill on May 23 with fever and muscular ache, her temperature rising quickly to 40—41° C On May 27 a few pinheadsized maculopapules appeared high up on the chest The patient died on the same day from circulatory failure and pulmonary oedema

The postmortem revealed a seriously hypertrophied heart with coronary sclerosis and myocardial fibrosis, stasis in other organs bronchitis and incipient pneumonia

The diagnosis of smallpox was verified by isolation of virus.

The case is not typable according to Dixon's scheme

Case 4 4 H B

Mrs H B was a 75 year-old woman who like the preceding patient E T (4 3) was in the same room as E W (3 4) during the period May 9—14 She was vaccinated as a child and revaccinated on May 16 (successfully) and was isolated on May 15 and three days later transferred to the Clinic for Infectious Diseases of the Danderyd Hospital She fell acutely ill on May 24 with fever rising rapidly during the next 24 hours to 41 C, but without other significant symptoms After three days maculopapules appeared simultaneously on arms hands and scalp and after another day on the trunk and face quickly changing to a vesiculopapulous exanthema which turned to pustules and scabs around May 31 The secondary fever lasted 11 days No complications arose apart from some thrombocytopenia for which the patient received thrombocyte enriched plasma on three occasions

The diagnosis was verified by isolation of virus

Dixon's type 6 discrete variola

Case 4 5 O G

O G was a 46-year-old building worker from Dalecarlia. He had come to Stockholm with his wife to attend the funeral of his sister in law D G (2 1) During the period April 28—May 8 he on several occasions

met his son K G (*3 2*) and his brother A G (*3 1*) with whom he stayed during his visit to Stockholm He received a primary vaccination on May 14 (successful), after smallpox had been discovered in his son and brother, and was isolated on the following day

On May 18 he fell ill with fever and pains in the back After five days eruptions appeared on chest and arms, spreading quickly on the following days By May 28 the whole skin was covered with efflorescences with pronounced oedema particularly on the face and extremities (Fig 26) He was highly febrile initially thereafter falling temperature up to May 28 with moderate secondary fever as from May 29 changing to septic peaks from June 7 onward Around June 1 signs of cardiac impairment ensued in the form of periods of fibrillation and a previously unrecognized cardiac murmur As from June 7 there were recurrent chills and fever peaks, up to 41° C and bronchopneumonia with ugly sputa from which Staph aureus, susceptible only to chloramphenicol, were cultured Death on June 15 O G was treated with Methisazone, procaine penicillin chloramphenicol, cloxacillin large doses of vaccinia immune globulin (although late in the course of the disease), digitalis and blood transfusions

The diagnosis was verified serologically and by isolation of virus

Dixon's type 4 benign confluent variola

O G's wife who had been exposed on the same occasions as her husband and had also received a successful primary vaccination on May 14 did not contract smallpox

Case 4 6 M H

Mrs M H was a 52 year-old nurse's assistant at the Clinic for Infectious Diseases of the Danderyd Hospital On the night between May 13 and 14 she nursed K G (3 2) She had been successfully vaccinated as a child and was revaccinated (successfully) within 24 hours of exposure She was isolated from May 15 On May 23 she fell acutely ill with headache and fever On the fourth day she was afebrile On the same day isolated papules or vesicles appeared in the mouth and pharynx, and on the face, back of the neck and upper part of the trunk The efflorescences fairly quickly pustulated or dried up

The diagnosis was verified serologically and by isolation of virus

Dixon's type 7 mild variola

Case 4 7, A A

Mrs A A was an 84 year old woman who lived with her daughter, M A (*3 10*) A A denied having had chickenpox but had been successfully vaccinated against smallpox in infancy She fell ill around May 28, feeling tired and unwell and with a headache Her temperature was not measured After some days a few eruptions appeared on the back of the neck and soon spread to the face, scalp, trunk extremities, palms and soles Efflorescences appeared also in the mouth and pharynx The exanthema changed in the normal way to vesicles, pustules and scabs (Fig 27) During the exanthematous stage she was febrile

The diagnosis was verified serologically and by isolation of virus

Dixon's type 6 discrete variola

A A had for several years suffered from pernicious anaemia and on June 6 she visited the medical outpatient department of the St Goran Hospital for a [illegible] of her blood disease W[illegible] red, she was [illegible] out-patient [illegible] In this way [illegible] nun[illegible] phy[illegible] the [illegible] tal [illegible] of s[illegible] over[illegible] been[illegible] ond[illegible]

Case[illegible]

Miss[illegible] nurs[illegible] the [illegible] ted[illegible]

nated on May 17 H M was probably infected on May 18 by the hospital worker S W (4 1) who transported food several times a day from the Hospital for Infectious Diseases to the Norrtull Hospital

She fell ill on June 3 with nausea vomiting and headache On the third day of her illness grain-of-rice-sized reddish papules appeared on the arms The head nurse interpreted her condition as a vaccination reaction H.M stayed at home from June 6 The exanthema spread quickly over the whole body but started to fade around June 13 She was isolated on June 23 when a small pox case (6 1) had been recognized in her ward On admission to the *Stockholm Hospital* for Infectious Diseases she had a few small scars on different parts of the body and on the hard palate

The diagnosis was made on the basis of clinical, epidemiological and serological data

Dixon s type 7 mild variola

Case 6 1 G.S

Miss G S was a 72 year-old woman who had been a patient for several years in Ward 3 at the Norrtull Hospital She was probably infected by the nurse s assistant H M (5 1) in that ward

On June 16 she fell ill with high fever (40 C) and a rash in the right axilla. The exanthema was interpreted as erysipelas On the same day she was given penicillin During the following days her temperature fell to 38 C The lesions in the axilla were now considered by the medical consultant to be zoster On June 18 and 19 a papulous exanthema appeared on the trunk thighs and face later spreading to the extremities A *dermatologist saw her on June 22* The patient was found to have lesions also on the soles and palms, typical of variola Apparently the initial exanthema in the axilla was not due to smallpox

The diagnosis was based on isolation of virus and serological tests.

Dixon s type 6 discrete variola

G.S in whose room there were 11 other women was transferred to an isolation room in the ward on the day she fell ill After suspicion of smallpox had arisen she was transferred on June 22 to the Hospital for Infectious Diseases She gave rise to two secondary cases of smallpox in the same ward (7 1 and 7 2)

Case 6 2 (M B)

Miss M B was an 85-year-old woman successfully vaccinated as a child She had been for a long time in Ward 3 at the Norrtull Hospital for schizophrenia She often came into contact with the nurse s assistant H M (5 1) who worked in that ward The patient was reported to have shown no symptoms of smallpox Serum samples were taken on June 23 from all patients in the ward for determination of the haemagglutination inhibition titre She was vaccinated (doubtful) on the same day and given immune globulin Her HI titre was found to be 1/160 and she was therefore admitted to the Stockholm Hospital for Infectious Diseases for check up The patient was subfebrile from June 28 to July 5 but was somatically free from symptoms Serum samples showed a significant rise in HI antibody titre Attempts to isolate virus from pharyngeal washings were unsuccessful

Not typable according to Dixon s scheme Variola asymptomatica

Case 7 1 A A

Mrs A A was an 88-year-old patient in Ward 3 of the Norrtull Hospital (senile dementia) She had been successfully vaccinated in infancy When the patients of the ward were vaccinated *on June 23* she was *considered* not to tolerate revaccination but was instead given 10 ml of immune globulin on that date and Methisazone 3 g twice daily from June 23 to 28

She fell ill on July 6 with moderate fever *lasting for two days* On July 8 a score of maculopapules of different sizes spread over the body were observed The efflorescences developed according to rule She had mild fever for five days On admission on July 8 she was *moderately dehydrated with some* rise of nonprotein nitrogen and had a bronchopneumonia She was *treated with*

met his son K G (*3 2*) and his brother A G (*3 1*) with whom he stayed *during his visit* to Stockholm He received a primary vacci nation on May 14 (successful), *after small* pox had been discovered in his son and *brother*, and was isolated on the following day

On May 18 he fell ill with fever *and pains* in the back After five days *eruptions* appeared on chest and arms, spreading quickly on the following days By May 28 the whole skin was covered with efflorescences with pronounced oedema particularly *on the face* and extremities (Fig 26) He was highly febrile initially thereafter falling *temperature* up to May 28 with moderate *secondary fever* as from May 29 changing to septic peaks from June 7 onward Around June 1 signs of cardiac impairment ensued in the form of periods of fibrillation and a *previ* ously unrecognized cardiac murmur *As from* June 7 there were recurrent chills and fever peaks up to 41° C and bronchopneu monia with ugly sputa from *which* Staph aureus, susceptible only to chloramphenicol, were cultured Death on June 15 O C was treated with Methisazone procaine peni cillin, chloramphenicol, cloxacillin large doses of vaccinia immune globulin *(although late in the course of the disease)* digitalis and blood transfusion.

The *diagnosis* was verified serologically *and by* isolation of virus

Dixon s type 4 benign confluent variola

O G s wife who had been exposed on the same occasions as *her* husband and *had also received* a successful primary vaccination on May 14 did not contract smallpox

Case 4 6 M H

Mrs M H was a 52 year old nurse s assist ant at the Clinic for Infectious Diseases of the Danderyd Hospital On the night be tween May 13 and 14 she nursed K G (*3 2*) She had been successfully vaccinated as a child and was revaccinated (successfully) within 24 hours of exposure She was isolated from May 15 On May 23 she fell acutely ill with headache and fever On the fourth day she was afebrile On the same day iso-

lated papules or vesicles appeared in the *mouth and pharynx*, and on the face, back *of the* neck and upper part of the trunk *The efflorescences* fairly quickly pustulated *or dried up*

The diagnosis was verified serologically *and by* isolation of virus

Dixon s type 7 mild variola

Case 4 7, A A

Mrs A A was an 84-year-old woman who lived with her daughter, M A (*3 10*) A.A denied having had chickenpox but had been successfully vaccinated against smallpox in infancy She fell ill around May 28 feeling tired and unwell and with a headache Her temperature was not measured After some days a few eruptions appeared on the back *of the neck* and soon spread to the face, *scalp* trunk extremities, palms and soles *Efflorescences appeared also in the* mouth and pharynx The exanthema changed in the *normal way to* vesicles, pustules and scabs *(Fig 27) During* the exanthematous stage *she* was *febrile*

The diagnosis was verified serologically *and by isolation* of virus

Dixon s type 6 discrete variola

A A had for several years suffered from pernicious anaemia and on June 6 she visited the medical outpatient department of the St *Goran* Hospital for a check up of her blood disease When *her* exanthema was observed *she* was referred to the dermatological out patient department of the same hospital In *this* way she came into contact with a large number of patients and staff Once the head physician of the dermatological clinic saw the patient she was transferred to the Hospi tal for Infectious Diseases on the diagnosis of smallpox *After* extensive investigations over 700 people were traced who might have been in contact with the woman No sec ondary cases occurred however

Case 5 1 H M

Miss H M 36 years of age worked as a nurse s assistant in a ward of 53 patients at the Norrtull Hospital She had been vaccina ted as a child and was successfully revacci

Vacc state at time of exposure[1]		Vacc after contact[4]	Immune globulin	Virus isol	Dixon type	Death	Source of infection
P in infancy	–	16 5 –			7		abroad
R 1955 56 59	?						
R 1960 61	+						
P 1920	?				1	23 4	1 1
P in infancy	?			16 5 –	6		1 1
P 1943	+			15 5 +	6		1 1
P 1950	+	16 5?			8		1 1
P in infancy	+	14 5 –		13 5 +	6		2 1
Not vacc				13.5 +	4	28 5	2 1
P in infancy	?	24 5 –			9		2 2
R 1918	?						
P 1916	+			15 5 –	6		2 2
P 1918	+			15 5 +	6		2 ?
P 1900	+			16 5 +	6		2 ?
R 1915	?						
P in infancy	+	24 5 –		15 5 –	9		2 3
R 1961	?						2 1
P in infancy	?	15 5 –			9		2 2
R 1936	+	20 5 –					
R 1962	+	27 5 –					
I 1950	–	17 5 –			8		2 ?
		21 5 –					
		25 5 –					
		27 5 –					
P in infancy	+	6 6 –		7 6 –	7		2 1
		13 6 –					
P 1949	+	14 5 +		21 5 +	7		2 2
							3 1
Not vacc		15 5 +	15 5 5 ml	24 5 +	6		2 8 3.9
P in infancy	+	16 5 +		28 5 +	Not typable	27 5	3 4
I in infancy	+	16 5 +		28 5 +	6		3 4
Not vac		14 5 +		27 5 +	4	15 6	3 1
							3.2
P in infancy	+	14 5 +		28.5 +	7		3 2
P in infancy	+			6 6 +	6		3 10
P in infancy	+			23 6 +	7		4 1
R 17 5 –63	+						
P in infancy	+			22 6 +	6		5 1
P in infancy	+	23 6?	23 6 5 ml		Variola asympt		5 1
P in infancy	–		23 6 10 ml	8 7 –	8		6 1
P in infancy	+	23 6 –	23 6 10 ml	7 7 +	7		6 1

This patient also got 3 g Methisazone twice daily during June 25—28

TABLE 7 Basic data on Smallpox cases in Stockholm 1963

No	Sex	Initials	Profession	Age	Date of exposure	Date of onset	Isolation[1]
1 1	♂	L O	Seaman	23	22–23 3	6 4	15 5 A
2 1	♀	D G	Home visiting nurse	57	6–11 4	18 4	23 4 D
2 2	♀	A W	Housewife	80	6–11 4	21 4	15 5 A
2 3	♀	M M	Clerk	23	16–25 4 6–11 4	25 4	15 5 D
2 4	♀	U M	Clerk	19	16–25 4 6–11 4	18 4[2]	15 5 A
3 1	♂	A G	Bricklayer	52	16–18 4 18–23 4	3 5	14 5 D
3 2	♂	K G	Bricklayer	19	16–17 4	5 5	14 5 D
3 3	♀	K P	Home visiting nurse	66	19 4 24 4	8 5	14 5 D
3 4	♀	E W	Shop assistant	55	26 4 23 4	6 5	15 5 D
3 5	♀	A F	Housewife	50	26 4 26 4	5 5	14 5 D
3 6	♀	E A	Housewife	71	21–27 4	8 5	16 5 D
3 7	♂	K C	Bricklayer	21	25 4–11 5	11 5	15 5 D
3 8	♀	R S	Hospital nurse's assistant	44	27 4–9 5	9 5	18.5 A
3 9	♀	E O	Hospital nurse's assistant	22	27 4–10 5	10 5	15 5 D
3 10	♀	M A	Layer out	53	26 4	8 5	7 6 A
4 1	♂	S W	Hospital worker	60	27 4–18 5	18 5	21 5 D
4 2	♀	I L	—	1	9–19 5	19 5	14 5 B
4 3	♀	E F	Housewife	72	9–14 5	23 5	15 5 B
4 4	♀	H B	Housewife	75	9–14 5	24 5	15 5 B
4 5	♂	O G	Building worker	46	3–8 5	18 5	15 5 B
4 6	♀	M H	Hospital nurse's assistant	52	13–14 5	23 5	15 5 B
4 7	♀	A A	Housewife	84	8–28 5	28 5	6 6 D
5 1	♀	H M	Hospital nurse's assistant	36	18 5	3 6	23 6 A
6 1	♀	G S	(Mental patient)	72	3–5 6	16 6	22 6 D
6 2	♀	M B	(Mental patient)	85	3–5 6		23 6 D
7 1	♀	A A	(Mental patient)	88	16–22 6	6 7	23 6 B
7 2	♀	R A	(Mental patient)	48	16–26.6	3 7	26 6 B

[1] B before D=during A=after infectious period

[2] Vaccinations. P=primary vacc R=revaccination, + =successful, — =unsucc[illegible]











Fig 18 Case *3 1* A G Age 53 Discrete variola (late stage)
Fig 19 Case *3 2* K G Age 19 Benign confluent variola (early stage pustules developing in eruptions of febrile mucocutaneous syndrome)
Fig 20 Case *3 2* K G Age 19 Benign confluent variola (late stage
Fig 21 Case *3 4* E W Age 55 Discrete variola
Fig 22 Case *3 5* A F Age 50 Discrete variola

fluid parenterally and penicillin and rapidly improved.

The diagnosis was verified by isolation of virus and serologically.

Dixon's type 8 abortive variola.

Case 7 2. R.A.
Miss R.A. was a 48-year-old patient with schizophrenia in Ward 3 of the Norrtull Hospital. She had been successfully vaccinated as a child. She was a room-mate of G.S. (*6 1*) when the latter fell ill with smallpox on June 16. On June 22 she was transferred to the Hospital for Infectious Diseases on account of a smallpox-like efflorescence on the medial side of the right knee. She was again in contact with G.S., (*6 1*) in the ambulance as well as at the Hospital for Infectious Diseases and was given 10 ml of immune globuline simultaneously.

R.A. was revaccinated on June 23 without success.

She fell ill on July 3 with fever. Four days later a maculopapulous exanthema appeared, changing into vesiculopustules and, later, scabs. The exanthema was discrete with a score of efflorescences spread over all parts of the body, especially on the face. During the exanthematous stage she had a successively falling fever lasting about a week.

The diagnosis was verified by isolation of virus.

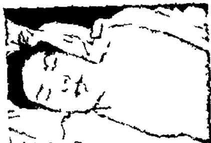
Dixon's type 7 mild variola.

Reference

1 Dixon C. W. Smallpox. London 1962.

18





19

20





21

22



F g 18 Case 3 1 A G Age 53 Discre e ar ola la e stage)

F g 19 Case 3 2 K C Age 19 Benign con fluen ar o a early stage pus ules de elop ng n erup on of febrile mucocu aneous syn a on e

F g 20 Case 3 K G Age 19 Benign con fluen ar ola late s age

F g 21 Case 3 4 E W Age 55 D scre e ar la

F g 22 Case 3 5 A F Age 0 Dis e e var ola

fluid parenterally and penicillin and rapidly improved

The diagnosis was verified by isolation of virus and serologically

Dixon's type 8 abortive variola

Case 7 2, R 1

Miss R A was a 18 year-old patient with schizophrenia in Ward 3 of the Norrtull Hospital She had been successfully vaccinated as a child She was a room mate of G S (*6 1*) when the latter fell ill with smallpox on June 16 On June 22 she was transferred to the Hospital for Infectious Diseases on account of a smallpox like efflorescence on the medial side of the right knee She was again in contact with G S, (*6 1*) in the ambulance as well as at the Hospital for Infectious Diseases and was given 10 ml of immune globuline simultaneously

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She fell ill on July 3 with fever Four days later a maculopapulous exanthema appeared, changing into vesiculopustules and, later, scabs The exanthema was discrete with a score of efflorescences spread over all parts of the body, especially on the face During the exanthematous stage she had a successively falling fever lasting about a week

The diagnosis was verified by isolation of virus

Dixon s type 7 mild variola

Reference

1 Dixon C W Smallpox London 1962











Fig. 23 Case *36* F. A. Age 71 Discrete variola.
Fig. 24 Case *41* S. W. Age 60 Mild variola.
Fig. 25 Case *42* I. C. Age 1 1/2 Discrete variola.
Fig. 26 Case *45* O. G. Age 46 Benign confluent variola.
Fig. 27 Case *47* A. A. Age 85 Discrete variola.

Clinical Survey

From the Hospital for Infectious Diseases Stockholm

Diagnosis, Clinical Classification and Symptoms, Differential Diagnosis and Therapy

JUSTUS STROM PER GERZEN, HARRY HERZENBERG, ULF JANSSON JAN URSING and HANS WERNEMAN

The diagnosis of smallpox may be based on the epidemiological conditions clinical symptoms and on virological and serological tests The clinical picture may be so characteristic that the diagnosis is certain On the other hand the disease has many facets and is modified and mitigated to a great extent by past vaccinations Detection of the presence of virus by cultures on the chorioallantois of chicken embryo or in tissue culture and also of specific antigen from vesicle contents or scabs by complement fixation reaction with antivaccinia serum is therefore of the greatest significance Guarnieri bodies can also be observed by trained investigators by microscopic analysis of scrapings from eruptions

The diagnosis can also be corroborated by serology through the finding of a high and rising titre of complement fixing neutralizing or haemagglutination inhibiting (HI) antibodies We attempted to make use particularly of the latter in this outbreak even though these tests are difficult to assess since vaccination produces the same reaction During an epidemic with the mass vaccinations that are then performed the value of serological tests is accordingly very limited In one case however we considered it justified to base the diagnosis on the epidemiological conditions and the HI titre

On occasions the diagnosis must be decided by the epidemiological analysis It may happen that the illness has been very mild with negligible abortive eruptions or has not come under medical supervision until all eruptions have disappeared yet the case must nevertheless be a link in the spread of the disease There is also a *variola sine eruptione* in which only the prodromal stage occurs and exanthema never develops

Finally one can gain some corroboration from vaccination with extra potent vaccine following an uncharacteristic

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On occasions the diagnosis must be decided by the epidemiological analysis It may happen that the illness has been very mild with negligible, abortive eruptions or has not come under medical supervision until all eruptions have disappeared yet the case must nevertheless be a link in the spread of the disease There is also a *variola sine eruptione* in which only the prodromal stage occurs and exanthema never develops

Finally one can gain some corroboration from vaccination with extra potent vaccine following an uncharacteristic

TABLE 8 Clinical classification of the smallpox cases in Stockholm 1963 (Dixon s classification 1—9)

Total	Name	Lethality (%)	Number	Deaths
1	Fulminating (Purpura variolosa)	100	1	1
2	Malignant confluent	70	—	
3	Malignant semi-confluent	25	—	
4	Benign confluent	20	2	2
5	Benign semi confluent	10	—	
6	Discrete	2	10	0
7	Mild	0	6	0
8	Abortive	0	3	0
9	Variola sine eruptione	0	3	0
	Variola asymptomatica		1	0
	Not typable		1	1
	Total		*27*	*4*

infection A certain significance can be attributed to this vaccination if negative

Since chicken pox often, and zoster occasionally, crop up in the differential diagnosis, it would be of value if the aetiological agent could be demon strated in these cases by cultivation This would be a positive, and not solely a negative finding through the absence of smallpox virus

Clinical classification of the smallpox cases

As noted, our patients were classified in accordance with the scheme proposed by Dixon in 1963, whose nine types are shown in table 8 We have added one case (*6 2*) of what we have called variola asymptomatica No smallpox symptom appeared in this patient, but she had the chance of being infected, her antibody titre was high and rising sharply, far above the level seen after vaccination All experience from other infectious diseases suggests that such cases must occur This is of more theo retical than practical significance since the infectiousness in such cases must be small

One of our cases (*2 1*) was of the fulminating type, which is always lethal But we had no representatives of the malignant types, which are characterized by haemorrhage in the skin and mucosa and a profuse, varying exanthe ma which develops slowly and never proceeds to pustulation Two of our patients (*3 2* and *4 5*) were of the benign confluent type, with hard pear-like efflorescences and pronounced pus-tulation These patients also died, as did the non typable case (*4 3*) who was suffering from heart disease and develop ed acute pulmonary oedema The re maining patients had the milder forms The discrete type usually shows typical development of quite numerous but nonconfluent efflorescences The mild cases have a fairly small number of

TABLE 9 Vaccination status and clinical type

Years since last vaccination	Type 1	2	3	4	5	6	7	8	9	Variola asympt	Not typable	*Total*
< 3							1		2			*3*
3 – 10												—
11 – 20						1	1	2				*4*
21 – 30												—
31 – 40							1					*1*
41 – 50	1					3	3	1	1			*9*
51 – 60												—
61 – 70						2					1	*3*
71 – 80						2				1		*3*
81 – 90						1						*1*
Not vaccinated				2		1						*3*
Total	*1*	—	—	*2*		*10*	*6*	*3*	*3*	*1*	*1*	*27*

efflorescences at most 100 the abortive at most 20 and no pustulation These cases also lack secondary fever The same applies to type 9 in which there is no exanthema We had no less than three cases of this type Finally there was one patient without any clinical symptom at all

The lethality was thus 4 out of 27 patients (15 %) a quite low figure

It is of interest to compare the clinical types with vaccination status (table 9)

The patient (*2 1*) who died of purpura variolosa had been vaccinated as a child more than 40 years previously — according to the record in the parish register The patient who died of cardiac insufficiency before smallpox developed had been vaccinated more than 60 years previously The two patients who died of the benign confluent form (*3 2* and *4 5*) were unvaccinated As regards the remaining 23 patients their vaccination status must be considered rather weak only three had been vaccinated in the past three years, the other 20 more than 10 years, and 16 of them more than 30 years previously But irrespective of whether vaccination had been done during the last few years or as long as 80 years and more ago the cases are uniformly distributed over the milder forms of the disease No conclusions of course, can be drawn from so small a material but it is noteworthy that the disease ran so lenient a course in most of the patients who had been vaccinated on some occasion

Some clinical symptoms and laboratory findings

The *prodromal stage* has a very great diagnostic significance The symptomatology is illustrated by figure *28*

The duration was between one and six days, average roughly four days One case was asymptomatic and thus

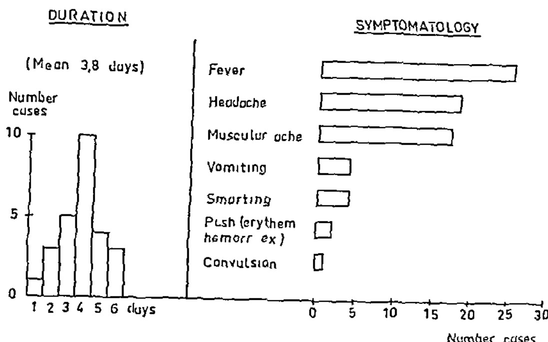


Fig 28

had no prodromal stage Fever, headache and muscular ache were the dominating symptoms — a picture reminiscent of influenza Vomiting occurred in four cases Smarting in the skin, which should be a characteristic feature, occurred only in 4 cases One patient had a rapidly disappearing eruption in the form of erythema, and another a widespread haemorrhagic exanthema with ulceration around the body orifices (Syndroma mucocutanea febrilis) The small child had generalized convulsions, a not unusual phenomenon of smallpox at the age

To arrange for consistent laboratory tests is obviously difficult in a smallpox epidemic In due course we succeeded in establishing a laboratory in the smallpox department and could also instal ECG and X ray apparatus Some of the findings are reported below

Liver Nine patients were examined in respect of transaminase Seven had altogether normal values of GOT and GPT The two lethal cases of benign confluent smallpox (3 2 and 4 3) on the other hand, had somewhat elevated values (100—200 units) one at the onset in conjunction with the febrile mucocutaneous syndrome the other towards the end of the illness

Kidneys Of 20 patients 14 had no pathological findings in the urine, and three only brief albuminuria The two aforementioned lethal cases and the patient with purpura variolosa had persistent albuminuria and haematuria The nonprotein nitrogen was also elevated in the two former one had a high

value from the start (60 mg %) which did not change. He had cystic kidneys, however. The other, his son, also had a high value from the start, but which rose continuously to 200 mg %. He too may have had congenitally affected kidneys.

Heart. In 13 of 20 cases investigated the ECG showed nothing abnormal. Five patients showed suspect changes, and the two cases of benign confluent variola definite signs of myocarditis. The two latter also had clinical signs of cardiac affection.

Lungs. X-rays were taken of 8 patients. There were pathological findings in patient *3.1* and in *4.5* who towards the end had staphylococcal pneumonia.

Blood picture. Anaemia developed in three patients: the two lethal cases and the child with discrete variola (*4.2*).

The white blood picture varied greatly according to the nature of the case. Leukocytosis might be present from the start, or the reverse might apply. An example of the former was patient *3.2* with benign confluent variola. During the first week he had 17 800—21 500 whites, which fell to 7 600—9 900 during the second week but rose again to above 21 000 in the third to fourth week. In case *4.4* with discrete variola the white cell count varied between 3 700 and 7 400 throughout. A marked feature was that irrespective of the cell count, both cases exhibited to a high degree a shift to the left. The eosinophile cells were usually still existent and were sometimes increased in number.

The thrombocytes could be fairly well followed in six cases. There was a fairly pronounced tendency to thrombocytopenia at the end of the prodromal or beginning of the exanthematous stage. Case *2.1* (purpura variolosa) had a count of 24 000 and case *3.2* (benign confluent) an extremely low count, 5 000, to some extent probably a reflection also of his incipient mucocutaneous syndrome of allergic origin. The thrombocytopenia could be a contributory cause of the haemorrhagic type of pustules. In all cases the thrombocyte count rose with development of exanthema and attained a normal level also in the lethal case *3.2*.

The sedimentation rate was often difficult to evaluate in the often elderly patients, who frequently exhibit high levels for other reasons. Judged by a few quite pure cases, the SR appears initially to lie between 30 and 50 mm/hr. In one case, however, it was only 10 mm.

Diseases of significance for differential diagnosis

During a smallpox epidemic people naturally become alarmed, especially by skin symptoms in conjunction with fever. Physicians are consulted by large numbers of people and it is understandable that under the stress of circumstances they often wish for expert advice. To prevent the hospital from being overrun a medical patrol was arranged, as already noted, which could visit patients in their homes before they were admitted to the hospital. A primary sifting of the material was obtained in this way, the number of suspect cases admitted being kept down to 67.

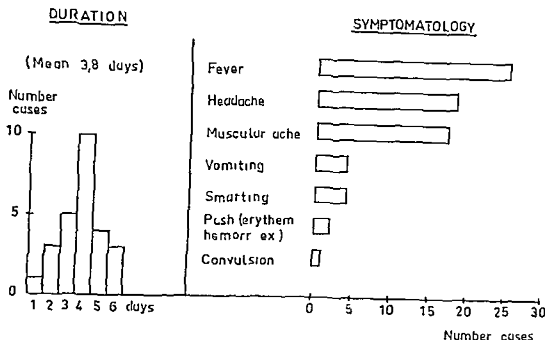


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Kidneys Of 20 patients 14 had no pathological findings in the urine, and three only brief albuminuria The two aforementioned lethal cases and the patient with purpura variolosa had persistent albuminuria and haematuria The nonprotein nitrogen was also elevated in the two former one had a high

Allergic exanthema was found also among the non contacts patients Of the five cases one was maculous one maculopapulous, one maculopustulous, one bullous exanthema and one erythematous.

Syndroma mucocutanea febrilis is of special interest in this context One of the smallpox cases (3 2) started with this syndrome which was evidently provoked by the infection If well developed the syndrome may resemble smallpox especially if the exanthema is of erythematous exudative multiform type The fever is often high and persistent This differential diagnosis is emphasized also by Dixon among others

The most common problem of differentiation is between *chicken pox* and smallpox Seven such patients were referred to us The following case may serve as an example The patient exhibited both the prodromal stage and suspect eruption

22 year-old woman Vaccinated and revaccinated Fell ill with headache and fever *On the third day she had itching eruptions* on face and scalp and temperature 39 4 She had pea sized maculopapules and thin walled vesicles containing transparent fluid over the whole body densest on the face where they were firm to hard She also had blisters on the gums and scleral conjunctiva and likewise on the palms of the hands and soles of the feet where they were also hard and long lasting She was highly febrile until the sixth day when the pustulation was most developed Complement fixation and virus cultures were negative

The two zoster patients illustrate that diagnostic difficulties may arise when the *zoster* is generalized

One was a 77 year-old woman who had been vaccinated She had been in contact with a shingles patient a fortnight *previously* Fell ill with fever and catarrhal symptoms She consulted a physician, who found eruptions on the trunk on the fifth day and fever of 38 8 *On admission to hospital she had* some 50 eruptions arranged in groups and consisting of grain-of rice sized reddened macules and small vesicles surrounded by a reddened *zone localized preferentially to* the neck chest and back with isolated vesicles also on the abdomen and extremities. Virus culture and complement fixation were negative

No less than six *cases of staphylococcal infection* in the skin were admitted on suspicion of smallpox, four of which were infected One such case was the following

21 year-old man Vaccinated as a child revaccinated on military service and now again *on June 17 1963 Six days later he had* spots on the legs which developed into purulent blisters On the eleventh day he was taken into the hospital when he was subfebrile and had a pea sized incrustated pock on the left upper arm He also had some 25 pinhead to bean sized pustules with surrounding redness and infiltration in the regions of the thighs seat and calves isolated pustules also on the neck and trunk Culture staphylococcus aureus Tests for vaccinia and smallpox were negative

The two patients with streptococcal and fungoid infection, respectively and reactive cutaneous lesions in the form of *bacterids* and *dermatophytids* are of interest These papulos cutaneous lesions, tending to vesicular and pustular formation appeared on the face and, particularly on the hands and feet The dermatophytids especially exhibited an extremely suspect picture in the form of lead shot like lesions on palms and soles

Table 10 Observation for variola

Disease	*Total*	Contacts	Non contacts
Allergic exanthema	*11*	6	5
Syndroma mucocutanea febrilis	*3*	—	3
Eczema	*3*	3	—
Chicken pox	*7*	—	7
Herpes zoster	*2*	—	2
Herpes simplex labialis	*2*	1	1
Purpura	*1*	1	—
Staphylococcal infection in the skin	*6*	2	4
Streptococcal infection with bacterids	*1*	—	1
Secondary infected mycosis with dermatophytids	*1*	—	1
Septicaemia with cutaneous metastasis	*1*	—	1
Pharyngitis, tonsillitis pneumonia, cystopyelitis	*6*	6	—
Febris incertae causae	*1*	—	1
Vaccination complications			
Without exanthema	*4*	4	
Secondary vaccinia	*2*	1	1
Post vaccinal exanthema	*15*	4	11
Zoster	*1*		1
Total	*67*	*28*	*39*

Apart from a clinical suspicion of smallpox there are two other circumstances which affect the indication for admission — the possible contact with a smallpox case and the vaccination history The cases admitted for observation are summarized in table 10

Twenty eight patients were contact cases Within this group, the clinical symptoms were often not so suspect As soon as any of the smallpox staff had fever, he or she was isolated Among them were the six cases in the table with diverse acute infections Thirteen patients in this group had eruptions, three in the form of eczema, one herpes simplex, one purpura, two staphylococcal infections and six allergic exanthema Of these allergic cases of exanthema, three were papulous two vesicopapulous and one maculopapulous haemorrhagic The following case is illustrative of this

M V 1 year 9 months Not vaccinated Had passed through an admission ward at a hospital in which a smallpox patient had been present Nine days later he was listless had eruptions on the scrotum and on the next day reddened grain of pepper sized papules on hands forehead thighs and buttocks After two days his temperature was 38 On admission he had some lead shotsized hard, partially incrustated papules on the aforementioned parts of the skin and two similar ones on the right palatal arch Virus isolation tests and complement fixation turned out negative for variola and vaccinia Diagnosis papulous urticaria

Allergic exanthema was found also among the non contacts patients Of the five cases one was maculous, one maculopapulous one maculopustulous, one bullous exanthema, and one erythematous

Syndroma mucocutanea febrilis is of special interest in this context One of the smallpox cases (3 2) started with this syndrome which was evidently provoked by the infection If well developed the syndrome may resemble smallpox, especially if the exanthema is of erythematous exudative multiform type The fever is often high and persistent This differential diagnosis is emphasized also by Dixon among others

The most common problem of differentiation is between *chicken pox* and smallpox Seven such patients were referred to us The following case may serve as an example The patient exhibited both the prodromal stage and suspect eruption

22 year-old woman Vaccinated and revaccinated Fell ill with headache and fever On the third day she had itching eruptions on face and scalp and temperature 39 4 She had pea sized maculopapules and thin walled vesicles containing transparent fluid over the whole body densest on the face where they were firm to hard She also had blisters on the gums and scleral conjunctiva and likewise on the palms of the hands and soles of the feet where they were also hard and long lasting She was *highly febrile until* the sixth day when the pustulation was most *developed* Complement fixation and virus cultures were negative

The two zoster patients illustrate that diagnostic difficulties may arise when the *zoster* is generalized

One was a 77 year-old woman who had *been vaccinated* She had been in contact with a shingles patient a fortnight previously Fell ill with fever and catarrhal symptoms She consulted a physician who found eruptions on the trunk on the fifth day and fever of 38 8 On admission to hospital she had some 50 eruptions arranged in groups and consisting of grain-of rice sized reddened macules and small vesicles surrounded by a *reddened zone localized preferentially to* the neck chest and back with isolated vesicles also on the abdomen and extremities Virus culture and complement fixation were negative

No less than six cases of *staphylococcal infection* in the skin were admitted on suspicion of smallpox four of which were *infected* One such case was the following

21 year-old man Vaccinated as a child *revaccinated on military service and now* again on June 17 1963 Six days later he had spots on the legs which developed into purulent blisters On the eleventh day he was taken into the hospital when he was subfebrile and had a pea sized incrustated pock on the left upper arm He also had some 25 pinhead to bean sized pustules with surrounding redness and infiltration in the *regions of the thighs* seat and calves isolated pustules also on the neck and trunk. *Culture staphylococcus aureus Tests* for vaccinia and smallpox were negative

The two patients with streptococcal and *fungoid infection, respectively* and reactive cutaneous lesions in the form of *bacterids* and *dermatophytids* are of interest These papulos *cutaneous lesions,* tending to vesicular and pustular formation appeared on the face and particularly on the hands and feet The dermatophytids especially exhibited an extremely suspect picture in the form of lead shot like lesions on palms and soles

TABLE 10 Observation for variola

Disease	*Total*	Contacts	Non contacts
Allergic exanthema	*11*	6	5
Syndroma mucocutanea febrilis	*3*	—	3
Eczema	*3*	3	—
Chicken pox	*7*	—	7
Herpes zoster	*2*	—	2
Herpes simplex labialis	*2*	1	1
Purpura	*1*	1	—
Staphylococcal infection in the skin	*6*	2	4
Strepto occal infection with bacterids	*1*	—	1
Secondary infected mycosis with dermatophytids	*1*	—	1
Septicaemia with cutaneous metastasis	*1*	—	1
Pharyngitis, tonsillitis pneumonia cystopyelitis	*6*	6	—
Febris incertae causae	*1*	—	1
Vaccination complications			
Without exanthema	*4*	4	
Secondary vaccinia	*2*	1	1
Post vaccinal exanthema	*15*	4	11
Zoster	*1*	—	1
Total	*67*	*28*	*39*

Apart from a clinical suspicion of smallpox there are two other circumstances which affect the indication for admission — the possible contact with a smallpox case and the vaccination history The cases admitted for observation are summarized in table 10

Twenty-eight patients were contact cases Within this group, the clinical symptoms were often not so suspect As soon as any of the smallpox staff had fever, he or she was isolated Among them were the six cases in the table with diverse acute infections Thirteen patients in this group had eruptions, three in the form of eczema, one herpes simplex, one purpura, two staphylococcal infections and six allergic exanthema Of these allergic cases of exanthema, three were papulous, two vesico papulous and one maculopapulous haemorrhagic The following case is illustrative of this

M V 1 year 9 months Not vaccinated Had passed through an admission ward at a hospital in which a smallpox patient had been present Nine days later he was listless had eruptions on the scrotum and on the next day reddened grain of pepper sized papules on hands, forehead thighs and buttocks After two days his temperature was 38° On admission he had some lead shotsized hard, partially incrustated papules on the aforementioned parts of the skin and two similar ones on the right palatal arch Virus isolation tests and complement fixation turned out negative for variola and vaccinia Diagnosis papulous urticaria

confluent cases (3 2 and 4 5) it was given after the exanthema had developed The cases nevertheless had a lethal outcome The drug was also used, again during the exanthematous stage on the child (4 2) with discrete variola In the fourth case (7 1) the treatment was started during the incubation stage She had a mild form of abortive variola This patient and the two lethal cases were also given immunoglobulin against vaccinia

No conclusions can, of course, be drawn from this small material but according to the manufacturer s statement the experience of treatment hitherto has not been favourable The situation is entirely different as regards prophylaxis, in which large scale trials in India have shown extremely good, not to say sensational results If these results are verified an important change may be expected in respect of our prophylactic measures in epidemics

The other main group — apart from four infected cases without exanthema — consisted of *postvaccinal complications* in the form of generalized eruptions. The largest number of cases, 15, had postvaccinal exanthema of the following types: urticarial 3, rubeoliform 1, papulous 5, papulopustulous 1, fine-vesicular 1, pustulous 1, exudative multiform 3. These may be illustrated by the following case.

29 year old man. Was vaccinated for second time on May 20, 1963. One vesicle appeared on the fifth day. From the 8th to 10th day his temperature was around 38°. On the 9th day he had red spots on the back. On admission to the hospital, apart from a small incrustated pock, he had exanthema consisting of roughly pea sized maculopapules moderately densely dispersed over the face, neck, chest, back, upper arms and hands. A number of the papules also showed central pustulation. Tests for vaccinia and variola were negative.

More unusual is that secondary vaccinia attains such a dispersion that the picture may be suspect, as in the following case.

40 year old man. Revaccinated on May 18, 1963 with normal take. On the 11th day his temperature was 39.2 and there was incipient exanthema. He was admitted to hospital on the next day. He had a heavy vaccination reaction on the arm and pea sized papulovesicules and papulopustules with surrounding inflammation dispersed singly on upper arms, thighs, forehead, upper and lower lip, back of the neck and upper part of the back, also two pea sized reddened macules above the palatal arches. Vaccinia virus was isolated from the efflorescences.

From the point of view of differential diagnosis one may say that careful study of the case history and clinical analysis, supplemented by bacteriological tests, should usually lead quickly to the correct diagnosis, but *must* be confirmed by negative virological tests for smallpox. A positive virological test may also be of value *in* cases of vaccination reaction, e.g. secondary or generalized vaccinia. It would also be desired to have positive support by demonstration of virus in cases of chicken pox and zoster. Serological tests may be of some help in unvaccinated cases, but seldom in vaccinated.

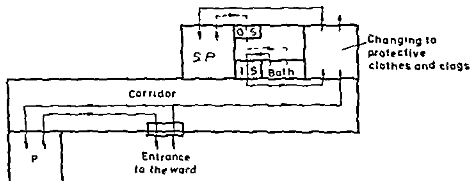
Therapy

General care and skin treatment are the primary considerations. Maintenance of the nutritional state of patients offered some problem in the severest cases with pronounced oral lesions but could sometimes be overcome with special nutritional solutions. The fluid and electrolyte balances were checked and any tendency to abnormality was corrected, and the acid base balance similarly. The skin treatment made very great demands, especially in the benign confluent cases. In one of these, secondary infection with staphylococcus aureus could not be avoided and pneumonia with the same aetiology ended the patient's life.

In the last few years a specific agent against smallpox virus had been prepared synthetically and tested under the designation 33T57 (methisazone) now called Marboran[R] (Wellcome, London). At our request this drug was immediately placed at our disposal. Four patients were treated with it. In the two benign

OBSERVATION WARD

(CARE OF SUSPECTED CASES OF SMALLPOX)



OS *Outer sluice*
IS Inner sluice
SP *Patients with suspected smallpox*
P Not suspected but possibly infected cases
—— Medical staff uninfected
-- *Medical staff infected*

Fig 29

in each ward was turned into a changing room From this room the visitor went out onto the terrace and thence into the patient s sealed room Leaving this room via the terrace he entered a sick room which had been reserved for undressing and bath. All clothing was left in the outer sluice (O S) Two paper sacks were placed in the sluice one for combustible materials (masks gloves) and the other for clothes to be disinfected The clogs were washed in 5 % chloramine and then left to air-dry After a shower in the adjoining bathroom he went into the inner sluice (I S) put on a protective coat and proceeded into the corridor to return to the changing room

Special rules were adopted for the care of patients in the observation wards and for dealing with objects in use Combustible material was employed as far as possible — syringes towels handkerchiefs etc In the outer sluice of the patient s room there was one paper sack for combustibles and one for laundry The sacks were sprayed with 5 % chloramine before being collected The yardmen in protective clothing then took the sacks on a special carriage The sacks were cut open when placed in the formalin fumigator Specimens for laboratory tests — scrapings from blisters and crusts blood samples etc — were transported in closed plastic bags wiped on the outside with chloramine (5 %)

When smallpox had been diagnosed and the patient was moved to the small pox pavilion the room was disinfected by thorough washing down with benzalkonium spirit

A separate pavilion was used for the care of patients with verified smallpox

Organization of Hospital Care for Smallpox Cases at the Hospital for Infectious Diseases in Stockholm

JUSTUS STRÖM

During the smallpox outbreak in 1963 the Stockholm Hospital for Infectious Diseases, comprising 450 beds, had two modern observation wards, with excellent isolation facilities, one rather old ward of similar type, seven separate buildings, each representing one ward, and a larger building with five wards, laboratories, etc

The patients are generally admitted to the observation wards for a preliminary diagnosis Patients who are seriously ill and those who cannot be placed among other patients because of infection risks, remain there The others are, according to diagnosis, removed to other wards

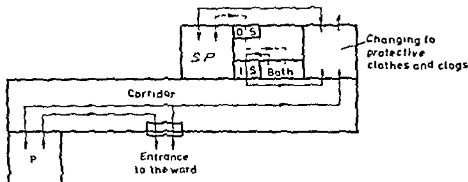
As soon as the first smallpox case in the Stockholm area (K G *3 2*) had been recognized, it was realized that two other smallpox cases (A W , *2 2*, A G , *3 1*) had been cared for in one of the observation wards (cf chapter I page 20) All other patients who had been in the same ward during that time or still were there, were regarded as possibly exposed and were therefore isolated In addition to these patients the three observation wards had to take all suspected cases of smallpox Once smallpox was verified, however, the patient was removed to a separate isolated pavilion

Another category of cases — the close contacts — was to begin with also admitted to the observation wards These cases were rather numerous, however, and soon two separate pavilions had to be reserved for them For the large number of other contacts isolation was provided by other means (cf chapter I page 22)

The contacts were cared for in the normal way with entrance to the rooms from the corridor of the ward, whereas the suspected smallpox cases were rigidly isolated in rooms sealed off from the corridor with entrance only from the outside of the building (Fig 29) On the least sign of possible smallpox symptoms, e g fever, in an isolated contact, the room was sealed off

With the exception of doctors and head nurses, separate personnel were used for the two types of rooms, sealed and not sealed, in the observation wards The most rigorous precautions were taken for the sealed rooms Before entering one of these rooms the visitor changed all clothes and put on an entire set of protective clothing, including clogs and disposable mask and gloves After the visit he changed again after a thorough shower, including a hair wash

The organization of this procedure is illustrated in Fig 29 The physician s room



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battery operation In this way the small pox department ultimately became a small hospital within the hospital

The selection of personnel for the care of smallpox cases and suspects may be a difficult problem as, naturally enough they are likely to have some terror of the disease At our hospital many members of the personnel had been exposed to mallpox through their contact with the first unknown cases All were revaccinated Those who pre viously had a satisfactory vaccinal state were put in charge of the care of patients with suspected or verified smallpox The others were isolated together with other contacts and were in charge of these isolation wards where everybody had been vaccinated before or at admission

A system for reliefs especially for the nurses and attendants in the small pox pavilion, was thought to be neces sary Originally it was planned to have a system of two preferably three days of continous duty followed by an equally long period off which would mean that one could manage with two or three teams But this proved to be difficult to arrange The question was solved by the personnel themselves Those who had worked in the ward from the start offered to continue despite all the strains, physical and mental involved Great assistance was rendered also by some of the patients relatives

The two head physicians of the hos pital together with four assistant physi cians took charge of both the smallpox pavilion the observation wards and the wards for isolated contacts They took up residence in the hospital, an arrange ment that was considered essential for practical and psychological reasons All personnel working in wards for suspected or verified smallpox cases had their meals in dining rooms separate from those of the rest of the hospital personnel, the staff of the smallpox pavilion actually in this ward

The remaining physicians were left in charge of the ordinary infectious disease work as well as the vaccination centre which was established at the hospital and of a rising number of patients with vaccination complications For the care of the latter patients two wards were reserved and an outpatient department was opened, at which the frequency of attendance was at times very high In this way the hospital was divided operationally as follows

1 *For smallpox cases*

A separate "hospital

2 *For smallpox suspects and contacts*

a) Three observation wards
b) One pavilion for close contacts from families of smallpox cases
c) One pavilion for contacts among the personnel of the ho pital
d) One pavilion for contacts among the patients of other hospitals

3 *For vaccinations*

One outpatient department

4 *For care of vaccination reactions and complications*

a) One outpatient department
b) Two wards

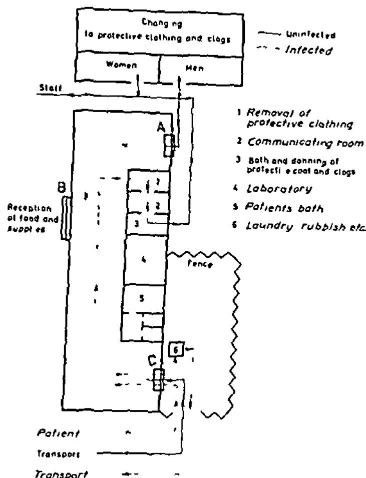


Fig 30

(Fig 30) Changing rooms for the personnel were arranged in an adjacent building After changing, the personnel walked across the yard and through a door (A) leading directly into the ward On leaving the ward all clothing was removed After a thorough shower they put on a protective coat and clogs, climbed out through the bathroom window, and returned to the changing rooms All food and equipment was delivered outside door (B) and was then collected by the ward personnel, all waste material, laundry etc, was taken out through door (C) in closed paper sacks sprayed with 5 % chloramine The smallpox patients also passed through door C Out of doors an area was shut off as a small park for the patients

A laboratory was soon arranged within the pavilion, in which not only routine analyses were made on blood and urine but also tests for fluid balance etc A laboratory trained nurse volunteered to do this work She also took her turn of duty as head nurse An electro cardiograph was installed, and finally also an X ray apparatus constructed for

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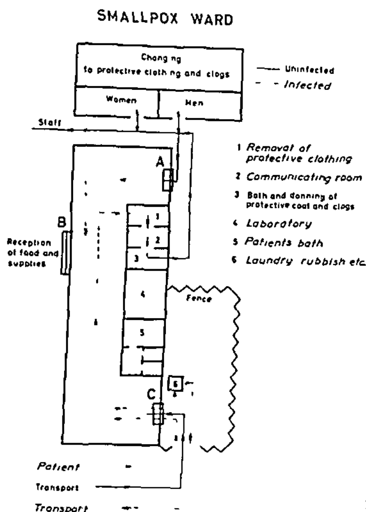


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Virological Investigations

From the National Bacteriological Laboratory Stockholm Sweden

Virological Findings During the Smallpox Outbreak in Stockholm in 1963

ÅKE ESPMARK, GUNNEL BIBERFELD, ASTRID FAGRAEUS, TORSTEN JOHNSSON, JONAS JONSSON and BIRGITTA MAGNUSSON

The rapid diagnostic treatment of specimens from cases suspected of smallpox is still one of the most urgent tasks of the virological laboratory Methodologically the prerequisites for fulfilling this demand are relatively favourable and this is also true in practice when only single samples are involved Difficulties which arise when large scale diagnostic work is required accordingly concern matters such as organization redisposition of localities and personnel training of auxiliary personnel supply of virus substrates antigens and sera etc When confronted by a smallpox outbreak it may be strongly felt that more planning and preparatory work ought to have been carried out in peacetime

One principal difficulty in smallpox diagnostic work originates from the serological identity of the variola and vaccinia viruses This means that the significance of antibody titers is seriously disturbed by postvaccinal titers shortly after extensive vaccination is started Accordingly growth characteristics of *isolated virus must be relied on for the* distinction between variola and vaccinia

Most of the data to be described below originate from the clinical material of the Stockholm area (Zetterberg et al (19) Strom et al (17) Bengtsson et al (1) A considerable number of specimens however, were received from other parts of Sweden For this reason the figures given below will not agree in detail with those found in the clinical reports in this issue

Material and methods

The virological diagnostic procedures, with the three main elements a) early diagnosis of variola b) virus isolation and c) serology were carried out according to conventional methods. The routine schedule followed is summarized in Figure 31 The methods will be described in some detail in the sequel

5 *For other infectious disease cases*
a) One observation ward
b) Three wards

In order to facilitate the rapid reorganization of the hospital and to reduce the initial anxiety and tension daily conferences were held in the mornings with all doctors and in the afternoon with the entire personnel At these conferences reports were given in the day by-day situation in the hospital and in Stockholm at large as well as on the actions taken or planned In the discussions that followed various questions were brought up that called for special investigation and action There is no doubt that these conferences were of the greatest importance

of a *rat hyperimmune serum* to vaccinia and 2 exact units of guinea pig complement The test was performed in serological tubes and 0 1 ml volumes of antigen, serum and complement were used A veronal buffer served as the diluent The mixture was incubated on a 37° C water bath for 1 hour before 0 2 ml of the haemolytic system was added (a 2 per cent suspension of sheep erythrocytes with 6 units of a rabbit amboceptor serum) Following this the tubes were again incubated at 37° C for 45 minutes Haemolysis was read against a standard scale and the antigen titer was calculated from the last tube showing less than 50 per cent haemolysis Two rows of test material checked against negative rat serum and diluent respectively, were included as controls A similar set of rows was set up with a *positive control antigen* from egg membranes infected with vaccinia virus as well as serum control tubes without any antigen

Virus isolation in eggs (3 5) Twelve days old eggs were preferred, but younger eggs (down to 10 days old) or older (up to 14 days old) were used, if necessary so that the continuous demand could be covered by two weekly deliveries of eggs A humidified hatcher adjusted to 36 C was available for the incubation of eggs

For the dropping of chorioallantoic membranes (CAM) a 2 × 3 mm hole was drilled in the shell about 1 cm away from the air sac and a further opening was made over the air sac by puncture with a needle A drop of prewarmed saline was placed over the uncovered shell membrane which was opened by gentle tangential pressure with a blunted needle The formation of the artificial air sac on suction over the natural air sac was controlled by candling

One tenth ml of the inoculum (test material undiluted and 1 1000) was applied to the CAM with a tuberculin syringe, both holes were closed with melted wax and the egg was returned to the humidified incubator for 2 or 3 days Four or six eggs were inoculated per dilution of the test material

For the harvest of an inoculated membrane the egg shell was cut longitudinally with scissors and the inoculated part of CAM stripped off with forceps and placed in a Petri dish. Readings for presence of pocks were performed against a dark background

Initially the findings on the membranes were matched against previously mounted type membranes with variola vaccinia and herpes simplex lesions

When required for passage or antigenic control, a clarified suspension was prepared from homogenates of harvested membranes

Virus isolation in tissue cultures

Roller tube cultures of trypsinized human embryonic or monkey kidney cells maintained in Parker's medium 199 were inoculated with 0 1 ml inocula and incubated at 36° C The tubes were read microscopically for cytopathic changes as a rule after 1 2 4, 6 and 10 days

The main interest concerned the distinction between variola vaccinia and herpes simplex virus degeneration on morphological grounds Fluid from degenerated cultures was tested in a HI

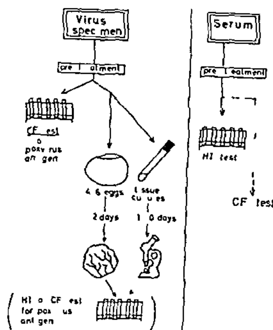


Figure 31 Virological d agnosis of smallpox Scheme of routine procedure followed

Specimens from skin lesions

Papules were scraped with a scalpel and the material was transferred to a sterile glass tube containing 1 ml of saline Drying pustules and scabs were handled similarly Vesicles and fresh pustules were punctured with a syringe containing 1 ml of saline and washed out by aspirating and injecting several times The walls of the pocks were cut with scissors and put in the same tube as the washing fluid It was recommended that material be taken from about 10 skin lesions but this was not always feasible When the specimen could be brought to the laboratory within 1 hour as was mostly the case no refrigeration was arranged Specimens sent from distant places by ordinary means of communication were kept cooled with common ice in thermos bottles

Upon arrival in the laboratory the specimen was handled in a sterile ventilated cabinet Pretreatment consisted of grinding tissue fragments together with washing fluid in a mortar without sand, addition of antibiotics (200 units of penicillin and 0 2 mg of streptomycin per ml) and centrifugation in rubber stoppered tubes at 2 500 RPM in an angle centrifuge for 10 minutes The clear supernatant was taken off and used as test material for antigen analysis (direct complement fixation test) and virus isolation No ether treatment of virus specimens (11) was performed

Other specimens for virus isolation

Throat specimens were obtained either as washing fluids or as swabs Such samples were pretreated with double amounts of antibiotics including fungistatics and were centrifuged for 20 minutes In some few instances heparinized blood spinal fluid or punctates from lymph glands etc , were sent in for virus isolation

Sera Blood samples (6—10 ml venous blood) were drawn without anticoagulant and sent in sterile rubber stoppered glass tubes The separated serum was heated at 60° C for 20 minutes and absorbed once with a one tenth volume of rooster erythrocytes before being tested Post mortem sera which had been shown to contain a high titer inhibitor (9) were absorbed with kaolin powder

Direct complement fixation test

The clarified test material in twofold dilutions from undiluted was subjected to a complement fixation test against constant amounts (4 antibody units)

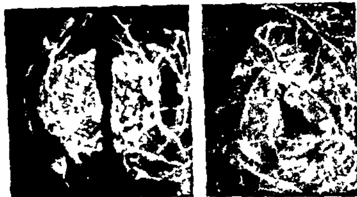


Figure 32 The appearance of variola vaccinia and herpes simplex pocks on the chorioallantoic membrane
(Left) Two halves of membrane with variola and vaccinia pocks respectively matched against each other
(Right) Membrane with herpes simplex pocks All membranes harvested 2 days after inoculation

vaccinia and herpes simplex lesions are wellknown from several textbooks and reviews (10 11 15) The essentials are briefly illustrated in Figure 32 The variola pocks are small mostly rounded and uniform in size vaccinia pocks are much larger and often umbilicated whereas herpes simplex lesions which may be of a similar size to variola pocks have a more irregular form and a *varying density especially in primary* isolate Difficulties may arise in differ*entiating variola and herpes simplex* if only very few or single pocks are *present on a membrane In such cases a* passage is mostly required for the final diagnosis

In cell culture (human kidney) the three mentioned viruses, if inoculated at moderate concentrations likewise form separate characteristic lesions (see Figure 33) Most pertinent is the difference between variola and vaccinia virus degeneration The variola lesions (plaques) consist of groups of rounded dense cells which stay on the glass so that the continuity of the monolayer remains relatively unbroken for several days Openings in the cell sheet are formed relatively late and even thereafter a large proportion of the degenerated (rounded) cells remain on the glass in a somewhat torpid manner

In the characteristic vaccinia plaque on the other hand most degenerating *cells are quickly detached from the* glass leaving a hole in the cell sheet *surrounded by relatively few rounded* cells The central opening is a pronounced *feature even of* very young vaccinia plaques

Virus isola ions from smallpox patterts

During the period from 13 May to 31 July, 380 specimens were received for virus isolation The samples were from cases of smallpox suspected smallpox and postvaccinal complications Most specimens consisted of material from skin lesions, but a considerable number

test against an antivaccinia serum (typing for poxvirus) or in a neutralization test against a known herpes simplex serum

Haemagglutination-inhibition test (HI test)

The *antigen* for HI tests consisted of clarified medium from HeLa cell cultures which had undergone rapid degeneration after inoculation of high titer vaccinia virus The antigen agglutinated erythrocytes from white Leghorn roosters to a titer of 1 32 to 1 64, read from bottom patterns in tubes

Twofold dilutions of the pretreated sera in saline, from 1 5, were prepared in serological tubes, pipettes being changed at each step To each tube 4 haemagglutinating units of antigen were added After about 30 minutes "sensitive" rooster erythrocytes were added to a final concentration of 0 25 per cent and readings were taken after another 45—60 minutes of incubation at 37° C One row with negative serum, and one with a known positive serum as well as an antigen titration and serum control tubes were included in each test Titers were expressed as the reciprocal of the last serum dilution that completely inhibited the agglutination

Complement fixation test (CF test)

The CF *antigen* was a clarified suspension obtained from homogenized liver tissue of newborn rabbits infected subcutaneously with vaccinia virus (13) The antigen had a CF titer of 1 160

Dilution series of sera were prepared as for the HI test except that a veronal buffer was used as a diluent To each tube were added 4 units of the antigen and 2 exact units of fresh guinea pig complement, and the mixtures were allowed to bind overnight at 4° C After adding the haemolytic system (stock suspension of 2 per cent sheep red cells with 6 units of rabbit ambo ceptor serum) to give a final erythrocyte concentration of 1 per cent, tubes were first incubated on a 37° C water bath for 30 minutes and then overnight at 4° C Tubes showing 50 per cent haemolysis or less were considered as positive Titers were expressed as the reciprocal of the initial serum dilution of the last positive tube Tests with control antigen from non infected liver tissue and controls for anticomplementary activity were included in each titration

Results

Early diagnosis by "direct complement fixation" test

All virus specimens from skin lesions were tested for the presence of complement-fixing poxvirus antigen immediately upon arrival As a rule the reaction was completed within 3 hours

Only specimens which later yielded positive virus isolations gave positive reactions with the direct CF test Of the 16 variola virus positive samples, 12 were positive in the CF test (75 %) The corresponding figure for 20 vaccinia virus positive samples was 4 (20 %) No false positive reactions were observed

Criteria for virus positive isolations in eggs and tissue cultures

The criteria by which variola pocks on egg membranes are distinguished from

TABLE 11 Source and number of specimens received for virus isolation May—July 1963 and number of samples yielding variola, vaccinia and herpes simplex virus. (Number of virus positive individuals within brackets)

Source of specimens	Number of specimens	Number of specimens (patients) from which virus was isolated		
		Variola	Vaccinia	Herpes simplex
Skin lesions and punctates	238	20 (16)	25 (25)	11 (8)
Throat swabs or washing fluids	89	4 (4)	1 (1)	—
Spinal fluid	15	—	—	—
Blood	17	—	—	—
Autopsy material	11	—	—	—
Total	*380*	*24 (16)*	*26 (26)*	*11 (8)*

of throat washings were also received as well as some specimens from other sources (see *Table 11*)

Specimens from the rash were obtained from 20 out of the 27 finally diagnosed smallpox cases Variola virus was isolated from 16 of these patients (see Table 12) From 3 of the 4 patients yielding negative results specimens were taken later than 18 days after onset of the disease All isolates of variola virus were obtained both in eggs and tissue cultures

As a rule a positive virus isolation could be reported to the respective hospital within 3 days after the arrival of the specimen (Table 12) In one case the specimen yielded a positive result only upon repeated attempts at isolation and in a few cases one passage was required to ensure the diagnosis On the other hand several fresh specimens were very rich in virus and produced confluent lesions of atypical appearance if inoculated undiluted on the CAM Accordingly two concentrations of each specimen were inoculated namely undiluted and 1 1000, which gave discrete lesions with either concentration in all positive cases

From four smallpox patients throat washings were taken at the time when skin lesions yielded positive isolations All throat washings were positive as well

Half the number of eggs were harvested after 2 days of incubation the rest after 3 days In no single case was the result of the 3-days readings different in principle from the finding after 2 days

Isolations of vaccinia and herpes simplex virus

Vaccinia virus was isolated from 26 patients and herpes simplex virus from 8 subjects during the period of the smallpox outbreak The source of specimens from which virus was obtained appears from Table 11 Most positive samples originated from vesicular eruptions distant from the vaccination pock in

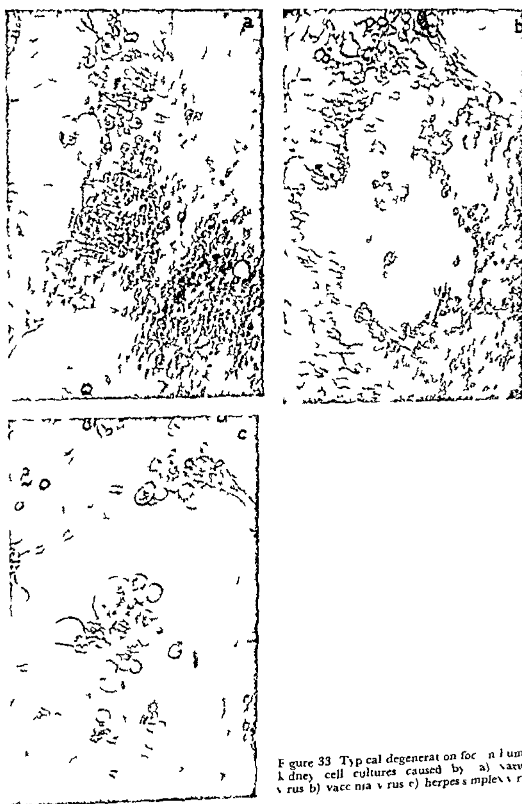


Figure 33 Typical degeneration foci in human kidney cell cultures caused by a) variola virus b) vaccinia virus c) herpes simplex virus

received during the acute phase of the outbreak After the epidemic the sera were subjected also to CF tests The diagnostic usefulness and simplicity of the HI test has been pointed out by several authors (4, 6, 12)

When attempts were made to sample a normal material representing antibody titers 4—6 weeks after successful vaccination considerable differences were found between different groups of individuals This is illustrated in Figure 34 The three histograms to the left represent from the top a frequently revaccinated population (laboratory personnel) a group of revaccinated old people in which the previous vaccination had been performed 50—75 years ago and finally a group of revaccinated conscripts, about 20 years old and not vaccinated since early childhood As seen the titer distribution is lowest for the frequently revaccinated group and highest for the old The three overlapping distributions were pooled to form the summarized distribution with a maximum frequency at 1 20 shown to the right in Figure 34 With due reservation for the heterogeneity mentioned this is taken to represent the antibody titers of an average population after uncomplicated vaccination

The HI antibody level in sera from smallpox cases [Strom et al (17)] and from hospitalized patients with postvaccinal complications [Bengtsson et al (1)] are graphed in Figure 35 a) in which the above normal material is included as a reference As seen most variola titers are of the magnitude 1 80 to 1 320 i e titer values shared by a considerable proportion of the

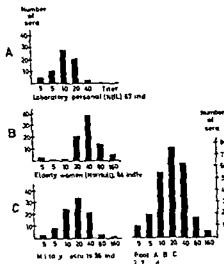


Figure 34 Distribution of haemagglutination inhibition titers (HI titers) against vaccinia antigen in normal populations about 5 weeks after vaccination

subjects with postvaccinal complications Apparently these late HI titers (like other late antibody titers) are of no great value in separate cases for the distinction of variola from postvaccinal conditions At the best they may carry some diagnostic information if combined with other data

It is of some interest to note that among the postvaccinal complications the titers were on the average significantly higher then in the normal material The possible implication of this will be discussed below

The complement fixation titers in the three groups are similarly presented in Figure 35 b The trend is approximately the same as for the HI titers as far as differentiation between variola and vaccinia infection is concerned

The rise and drop in HI titers in relation to time is shown in Table 13

TABLE 12 Early virological findings in the 27 cases finally diagnosed as smallpox (N D = not done, no specimen available)

Patient	Virus isolation: Result (16 positive)	Virus isolation: Days after receipt of specimen when positive isol was reported	CF test for poxvirus antigen (12 positive)	HI titer of the 1st serum sample obtained
LE O	ND		ND	1 320*
D G †	ND		ND	ND
A W	neg		neg	1 80*
M M	+	3	neg	1 320*
U M	ND		ND	1 80*
A G	+	2	1 64	1 1 280*
K G	+	2	1 32	1 40*
K P	ND		ND	1 160*
L W	+	3	1 2	1 320*
A F	+	2	1 2	1 320*
E A	+	2	1 64	1 80*
K C	neg		neg	1 40*
R S	ND		ND	1 80
T O	ND		ND	1 40*
M A	neg		neg	1 80
S W	+	4	1 2	1 5
I L	+	5	1 16	1 5
F T	+	2	1 2	ND
H B	+	2	neg	1 40
O G	+	2	1 2	1 20
M H	+	14	neg	1 160
A A	+	2	1 2	1 40
H M	neg		neg	1 80
G S	+	2	1 4	1 160
M B	ND		ND	1 40
A Å	+	3	1 4	1 10
R A	+	4	neg	1 20

* Sera drawn within 3 days after initial diagnosis of the outbreak

recently vaccinated individuals It is worth noting that specimens from the common atypical postvaccinal rashes were negative, as were also spinal fluids from cases of postvaccinal neurological complications

Serological findings

Comparison between variola titers and postvaccinal titers

The haemagglutination inhibition test (HI test) was used for antibody titration of about 3,000 serum samples

received during the acute phase of the outbreak After the epidemic the sera were subjected also to CF tests The diagnostic usefulness and simplicity of the HI test has been pointed out by several authors (4, 6, 12)

When attempts were made to sample a normal material representing anti body titers 4—6 weeks after succes ful vaccination considerable differences were fourd between different groups of individuals This is illustrated in Figure 34 The three histograms to the left represent from the top a frequently revaccinated population (laboratory personnel) a group of revaccinated old people in which the previous vaccina tion had been performed 50—75 years ago and finally a group of revaccinated conscripts about 20 years old and not vaccinated since early childhood As seen, the titer distribution is lowest for the frequently revaccinated group and highest for the old The three overlapping distributions were pooled to form the summarized distribution with a maximum frequency at 1 20 shown to the right in Figure 34 With due reservation for the heterogeneity men tioned this is taken to represent the antibody titers of an average population after uncomplicated vaccination

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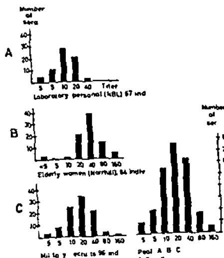


Figure 34 Distribution of haemagglutination inhibition titers (HI titers) against vaccinia antigen in "normal populations about 5 weeks after vaccination

subjects with postvaccinal complications Apparently these late HI titers (like other late antibody titers) are of no great value in separate cases for the distinction of variola from postvaccinal conditions At the best they may carry some diagnostic information if combined with other data

It is of some interest to note that among the postvaccinal complications the titers were on the average signif icantly higher then in the normal material The possible implication of this will be discussed below

The complement fixation titers in the three groups are similarly presented in Figure 35 b The trend is approximately the same as for the HI titers as far as differentiation between variola and vac cinia infection is concerned

The rise and drop in HI titers in relation to time is shown in Table 13

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A W	neg		neg	1 80*
M M	+	3	neg	1 320*
U M	ND		ND	1 80*
A G	+	2	1 64	1 1 280*
K G	+	2	1 32	1 40*
K P	ND		ND	1 160*
I W	+	3	1 2	1 320*
A T	+	2	1 2	1 320*
L A	+	2	1 64	1 80*
K C	neg		neg	1 40*
R S	ND		ND	1 80
E O	ND		ND	1 40*
M A	neg		neg	1 80
S W	+	4	1 2	1 5
I L	+	5	1 16	1 5
L T	+	2	1 2	ND
H B	+	2	neg	1 40
O G	+	2	1 2	1 20
M H	+	14	neg	1 160
A A	+	2	1 2	1 40
H M	neg		neg	1 80
G S	+	2	1 4	1 160
M B	ND		ND	1 40
A Å	+	3	1 4	1 10
R A	+	4	neg	1 20

* Sera drawn within 3 days after initial diagnosis of the outbreak

recently vaccinated individuals It is worth noting that specimens from the common atypical postvaccinal rashes were negative, as were also spinal fluids from cases of postvaccinal neurological complications

Serological findings

Comparison between variola titers and postvaccinal titers

The haemagglutination inhibition test (HI test) was used for antibody titration of about 3,000 serum samples

TABLE 13 D stribution with time of HI titers in smallpox patients and cases of postvaccinal com plications

Titer										
23 smallpox patients (91 sera)	2 560				1					
	1 280				1					
	640				—	1	1			
	320		2	5	1	2	2			
	160	3	5	6	3	3	4	1		1
	80	1	5	5	2	4	1	2	1	4
	40	4	1					1	3	1
	20	1							1	1
	10	6								
	5	2								
	<5	3								
Week		1	2	3	4	5	6	7 9	Month 3	Month 4
Number of sera		20	13	16	8	10	8	4	5	7

Titer										
149 postvacc compl c (347 sera)	640				1	1				
	320		2	2	2	3		1		
	160		4	9	7	6	3	—		
	80		15	9	19	16	9	4	2	
	40		19	12	8	11	15	9	7	2
	20		21	5	6	4	3	4	4	5
	10	2	18		2	—	1	1	2	14
	5	3	8					—		[illegible]
	5	10	26	2	—	—	—	—	2	1
Week		1	2	3	4	5	6	7–9	Month 3	Month 4
Number of sera		15	113	39	45	41	31	19	17	27

during this period especially the HI titers Of 20 sera drawn from smallpox patients during the first six days after onset 15 had a HI titer of 1 10 or higher whereas none of 9 sera taken within 6 days after vaccination attained this titer The CF titers seem to rise some what more slowly than the HI titers but later this difference becomes less pronounced An estimation of the rela tive advantage of the two serological reactions may be derived from the corre lation diagram of Figure 37 Most points in the left hand part of the diagram (with relatively high HI titers and low CF titers) *represent early* sera For relatively late sera (most of which are in the upper right portion of the diagram) it seems, on the other hand, that the CF titers may better differentiate between variola and vaccinia

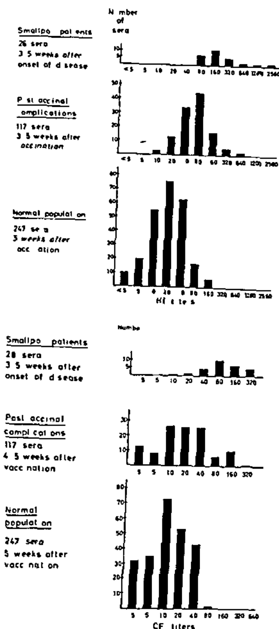


Figure 35 a Distribution of haemagglutination inhibition titers in sera drawn 3—5 weeks after onset of infection from smallpox patients cases of postvaccinal complications and from a normal population

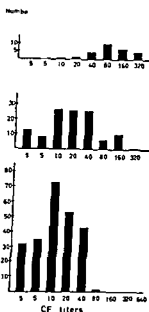


Figure 35 b Distribution of complement fixing antibody titers in sera drawn 3—5 weeks after onset of infection from smallpox patients cases of postvaccinal complications and from normal populations

At least the postvaccinal titers decrease fairly rapidly — to an average titer of 1 10 in the 4th month This conforms with the well known experience that old vaccination titers are practically always low, and it is this that constitutes the usefulness of the *HI* test in the initial phase of a smallpox outbreak before extensive vaccination has yet been started

The development of antibody titers during the first few days after onset of smallpox or after vaccination is accordingly of major interest for the diagnostic evaluation of HI and CF tests in the initial phase of a smallpox outbreak *A detailed presentation of the time* titer relationship during the first 11 days is shown in Figure 36 As a whole, variola and vaccinia titers are separated

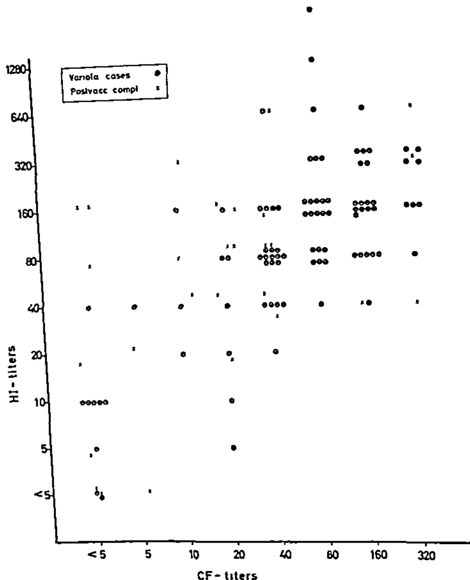


Figure 37 Correlation between complement fixation (CF) titers and haemagglutination inhibition of sera from cases of smallpox and postvaccinal complications

smallpox patients in whom the spread of antigens is known to be very extensive

b) High antibody titers and complications might instead be associated through the existence of a predisposed hyperreactivity of certain individuals This would require the assumption of an increased reactivity of *both* antibody

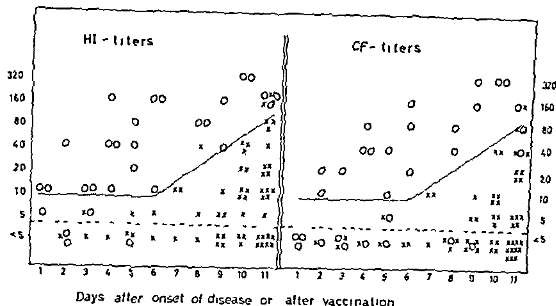


Figure 36 The early development of antibody titers among smallpox cases (circles 31 sera) and postvaccinal complications (crosses 59 sera)

Antibody titers of patients with postvaccinal complications

As mentioned, the HI titers among cases of postvaccinal complications were on the average significantly higher than in the vaccinated normal material The same holds, although to a lesser degree, for the CF titers The differences between means are shown in a condensed form in Figure 38 The discrepancy could only partly be ascribed to differences in the earlier vaccination history As regards diagnostic work this means that a titer distribution derived from individuals after uncomplicated vaccination may not be an adequate reference for the distinction between postvaccinal titers and smallpox titers In practice differential diagnostic problems often concern recently vaccinated people with possible smallpox contact who develop hyperpyrexia, generalized rashes, secondary pocks, etc The "normal" postvaccinal titer reference should therefore be based on titers connected with severe reactions and complications This principle was followed in the above evaluation of the diagnostic significance of serological reactions

The mentioned differences of titer levels may possibly have some bearing upon the pathogenesis of complications in general

a) High antibody titers and complications may both be due to the dissemination of abnormally large quantities of antigens in the organism

As reported by Bengtsson et al (1) the severity of local reactions was positively correlated to the HI titers (but not to CF titers) within the total complication material, whereas equal titers were found in subgroups representing different forms of complications It is furthermore well known that the antivaccinia titers are still higher in

rom the diagnostic procedure under ordinary circumstances. It is true that variola and vaccinia viruses can be confidently distinguished also in tissue cultures but this requires some degree of training In the isolations on eggs incubation for 3 days gave no additional information as compared with results after 2 days On the contrary 2 days incubation seemed preferable since the risk of confluent lesions with heavy inocula was less The early diagnostic test (i e direct complement fixation test) gave positive results in 75 per cent of the virus positive case The CF negative specimens were those which also yielded relatively few pocks in virus isolations In this respect the CF test results were considered satisfactory As regards serological reactions it should be pointed out that the HI test is of great diagnostic value in times when recent vaccination is not commonly encountered (see last column of Table 12) HI titers attain a significant level 5—7 days after onset of the disease and old vaccination titers are low The first two cases subjected to laboratory investigation during this outbreak had HI titers of 1 1260 and 1 40 These significant results were obtained two hours after arrival of the blood samples

During the acute phase of the outbreak shortage of time due to the large number of specimens obtained prevented the introduction of methods not previously tested at the laboratory The lack of reliable methods for positive varicella diagnosis was markedly felt Cytological investigation on smears of vesicle material (2) was attempted with some success in single cases as was virus isolation in human cells For future needs practical training in and evaluation of the cytological test or alternative rapid tests (e g antigenic analysis by CF test (16)] should be done in advance

The same holds for newer methods of early smallpox diagnosis reported to have yielded better results than the antigen analysis by the CF test Dumbell and Nizamuddin reported good results with a modification of the diffusion in gel technique (7) Peters et al (14), working with early smallpox diagnosis by electron microscopy claimed to have obtained results comparable in sensitivity to virus isolation in eggs Before this advanced technique becomes more generally available it may be wise to incorporate the direct light microscopy of stained smears as an early test This old method seems to have attracted new attention in later years (18)

However in attempts to perfect the speed of smallpox diagnosis, it may be worthwhile to consider that much more time can generally be saved by moderate improvements in the clinical and epidemiological field than in the virus laboratory

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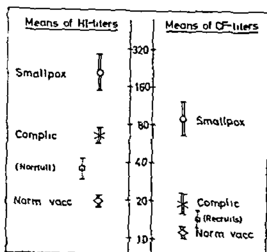


Figure 38 Mean values of HI and CF titers about 1 month after onset of infection in a group of smallpox patients, among cases of postvaccinal complications and in a normal vaccinated population Standard errors of the means are given as 95 % confidence intervals Also included are the partial populations (Norrtull and Recruits) which contribute the highest titer distributions in the normal material (cf Figures 34, 35 a and 35 b)

producing cells and of other (different) immunological mechanisms which may form the basis of severe reactions and, at least, some complications

Evidently a coincidence of the two alternatives (a large antigen dose and predisposition), will occur in reality, constituting a comparatively large risk for complication A suggestion of the importance of the dose appears from studies showing a significant correlation between vaccine dosage and the severity of local and general reactions (8)

Discussion

As frequently experienced in the European smallpox outbreaks during latter years the disease is often introduced, and even spread, in an atypical form by persons with partial immunity Hence, much reliance has to be placed upon the virological laboratory for the rapid detection of initial smallpox cases Only moderate personnel and material resources are required for this first stage of preparedness

a) general facilities for handling ' highly infectious matter",

b) a promptly operative serological set for early diagnosis by antigenic analysis and by HI tests on sera (i e tested antigens, non crossing antisera, detailed working instructions etc),

c) immediate supply of 10—14 days old chick embryos,

d) personnel trained in egg technique (CAM) and the reading of membranes (distinction between variola, vaccinia and herpes simplex pocks),

e) tissue cultures (not essential)

Probably it would serve the efficacy of the epidemiological surveillance if such emergency resources were available at the regional laboratories Firstly, the time loss due to transport could be reduced and, secondly, the clinicians would probably send specimens more liberally if local laboratory facilities were ready at hand

For the central laboratory, which is supposed to carry out the large scale diagnostic work following the initial diagnosis, the advance planning mainly concerns a manifolding of the mentioned resources, supplemented by personnel on duty and a staff for auxiliary services such as sorting and registration of specimens writing, reporting, etc

Some few concrete experiences from the laboratory work during the Stockholm outbreak will be briefly mentioned It was felt that eggs cannot be omitted

from the diagnostic procedure under ordinary circumstances It is true that variola and vaccinia viruses can be confidently distinguished also in tissue cultures but this requires some degree of training In the isolations on eggs, incubation for 3 days gave no additional information as compared with results after 2 days On the contrary 2 days incubation seemed preferable since the risk of confluent lesions with heavy inocula was les The early diagnostic test (i e direct complement fixation test) gave positive results in 75 per cent of the virus positive cases The CF negative specimens were those which also yielded relatively few pocks in virus isolations In this respect the CF test results were considered satisfactory As regards serological reactions it should be pointed out that the HI test is of great diagnostic value in times when recent vaccination i not commonly encountered (see last column of Table 12) HI titers attain a significant level 5—7 days after onset of the disease and old vaccination titers are low The first two cases subjected to laboratory investigation during this outbreak had HI titers of 1 1280 and 1 40 These significant results were obtained two hours after arrival of the blood samples

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CHAPTER V

Vaccination Problems

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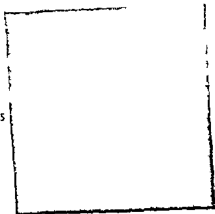
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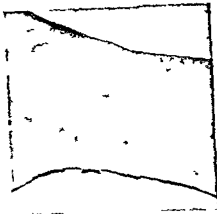


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F g 39 Strong local react on with erysipelaslike effl rescence of the upper ar n
F g 40 Pox on the th gh and suppurat ng regional lymphaden us n the gro n
F g 41 Lyn phang t s
F g 42 Suppura ng lymphad n s
F g 43 Urt caria
F g 44 Urt carial rash













F g 4 R bella like ra h
F g 46 Mul form rash on h e f rearm of the oculated arm
F g 47 Dense ves cular eruptions the ra h pared at he w stwa ch a ea
F g 48 Haemorrh ag c urticar al rash
F g 49 Herpes zos er ke rash
F g 50 Ecema vacc natum

From the Hospital for Infectious Diseases, Stockholm

Postvaccinal Reactions and Complications

ELIAS BENGTSSON, SVEN HANSSON and BERTIL NYSTRÖM

The voluntary mass vaccination performed in Stockholm in conjunction with the smallpox outbreak in 1963 was of an extent that can only be approximately estimated (52) In round figures 300 000 people must have been vaccinated in central Stockholm (about 1/2 million in the entire Stockholm area) within the course of a few weeks In the event of a severe reaction or complication the public were advised to report to their vaccinating physician or to the Hospital for Infectuous Diseases at which the treatment of such cases was centralized This centralization brought advantages in the form of uniform assessment accumulated experience and facilities for analysis of the results The series presented here is nevertheless selective It consists of patients who presented at the Out patient Department of the Hospital for Infectious Diseases in central Stockholm and of patients who were admitted to this hospital The object of this paper is to report on the reactions and complications observed and the mutual relations between them

A general description will be given of the series with the emphasis on local and generalized benign reactions and complications and their correlation both to routine clinical and laboratory observations and to serological reactions Allergological studies (46), circulatory and electrocardiographic postvaccinal reactions (6), neurological complications (31) and virological investigations (17) are reported in other papers

Material

The outpatient series comprises 884 cases, the clinical 192 they coincide only to a small extent The majority of persons in both groups were aged 15—60 years In the outpatient group only about one fourth consisted of elderly persons and children Females were twice as numerous as males (table 14)

This may obviously be due to the fact that a primary vaccination had been performed on females to a higher percentage than on males (26 against 14 per cent in the age group 15—60 years) as shown in table 15 This table shows, furthermore a high primary vaccination rate among children

Except in respect of diagnostic aspects the outpatient group is not included in the analyses of the inpatient material reported below

Methods

Patients admitted to the hospital for clinical treatment were placed in two of its wards and were kept continuously under the authors supervision On arrival and thereafter once a week, analyses were made of the peripheral

TABLE 14 Sex and age distribution

	Outpatients (884)	Inpatients (192)	*Total (1076)*
♂	275	74	*349*
♀	609	118	*727*
Below 15 years	97	42	*139*
15—60 years	656	119	*775*
Above 60 years	131	31	*162*

TABLE 15 Percentage of primary vaccinees by sex and age groups (outpatients only)

Age group	♂	♀	*Total*
Below 15 years	*90 5* (38/42)	80 9 (45/55)	*85 6* (83/97)
15—60 years	*14 3 (27/189)*	*26 0 (106/407)*	*20 3* (133/596)
Above 60 years	0 (0/44)	4 6 (4/87)	*3 1* (4/131)

blood picture and electrocardiograms were recorded, thrombocyte count, examination of serum transaminases (SGOT, SGPT) and paper electrophoresis were also done at admittance Acute-phase and convalescent phase sera were collected for determination of antibodies to vaccinia antigen by means of haemagglutination inhibition (HI test) and complement fixation (CF test) Titres are expressed as the reciprocal of the highest dilution of serum giving a positive reaction in the respective tests In cases of meningoencephalitis and of exanthema of, for example, rubella type, CF tests were performed against enterovirus In the same cases attempts were made to isolate virus from faeces, and in the meningoencephalitis cases also from cerebrospinal fluid Attempts at virus isolation were made in a limited number of cases on aspiration from vesicles and inflammatory tissue In some cases a histological examination was made of the tissue around the lesion Bacteriological cultures of lesion contents were performed in cases with severe suppurating lesions

Lumbar punctures were performed and electroencephalograms recorded on patients with headache, vomiting, nuchal rigidity or other neurological symptoms All cases exhibiting neurological manifestations were examined by the same neurologist Thirty one patients with allergic manifestations, particularly urticarial postvaccinal exanthema, and some patients with encephalitis and myopericarditis, were examined by an allergologist when the acute phase had passed All patients were readmitted to hospital a fortnight after discharge for control of clinical status and laboratory tests as required Every complication was followed up until it had healed or had remained unchanged for a lengthy period

The material was data processed and analysed by the chi square method with Yeate s correction (39)

TABLE 16 Percentage of complications and reactions after smallpox vaccination in outpatients and inpatients

	Outpatients (884)	Inpatients (192)
Erysipelas like efflorescences	23 7	13 5
Lymphangitis lymphadenitis abscess	26 3	12 0
Postvaccinal exanthema	21 1	25 9
Daughter lesions	14 1	9 0
Secondary pox	15 3	24 0
Encephalopathy meningitis and neuritis		7 3
Pericarditis and myocarditis		3 7
Generalized benign vaccinia		3 2
Eczema vaccinatum		3 7
Gangrenous vaccinia		1 0

TABLE 17 Number of cases of postvaccinal exanthema among outpatients and inpatients

	Inpatients	Outpatients	*Total*
Exanthema urticarial	17	49	*66*
Exanthema morbilliform	2	6	*8*
Exanthema scarlatiniform	1	1	*2*
Exanthema rubeoliform	3	13	*16*
Exanthema multiform	5	5	*10*
Nodose erythema	4	4	*8*
Mucocutaneous syndrome	1	1	*2*
Other types	16	38	*54*
Total number of cases	*49*	*117*	*166*

Results

Incidence and types of reactions and complications (Tables 16—17)

The observed reactions and complications were diagnosed largely according to the acknowledged pattern (9) and were classified as benign or malignant — the benign reactions which were chiefly treated on an ambulatory basis being divided into five main groups (Table 16) Every fourth or fifth patient ambulatory treated had erysipelas like efflorescences lymphangitis or postvaccinal exanthema Daughter lesions and secondary pox were also common

Severe local reactions (fig 39) were usual especially among the few cases vaccinated on the thigh (fig 40) Bacteriological cultures were performed in roughly half of the cases with very severe local reactions Growth of staphylococcus aureus was found in 25 cases, of streptococcus in 5 and of other bacteria in 4, in 62 cases no growth was found Bacteriological cultures were not performed in 100 cases Virus culture from the primary lesions was performed in 53 cases Vaccinia virus was discovered in 16 cases, but in the other 37 the culture was negative

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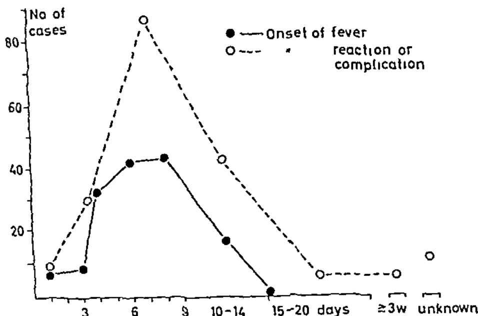


Fig 51 Onset of fever or complication

dominated Next came eczema vaccinatum and myopericarditis (7 cases each) A dissemination of lesions was diagnosed in six cases and a gangrenous form of vaccinia was considered with some hesitation to exist in two We found no case which could be definitely denoted as malignant generalized vaccinia

Among the cases of eczema vaccinatum only one was of threatening character This was a 20 year-old man with B snier s prurigo who was infected by his revaccinated mother His entire face and neck were covered with lesions which were also widespread on the upper part of the trunk (fig 50) He manifested neurological complications (31) and marked ECG changes later followed by serious hair loss and pigment changes, but apart from certain scars he had no lasting ill effect Other cases of eczema vaccinatum were comparatively mild

The two cases of probable gangrenous vaccinia had ulcerations extending down into the musculature but not deeper and without tendency to lateral progression even if the sores were large and persisted for several months

Onset and duration of fever and of reaction or complication (Fig 51 —52)

An overall calculation showed the fevers and reactions generally appeared on about the seventh day after vaccination the reactions, however, tending to come first Fig 51 reveals the well known fact that in the revaccinated the reactions appear already on the third to fourth

Violent local reactions with almost *erysipelas-like*, indurative efflorescences well delimited from the surrounding normal skin were seen in the outpatient series in no less than 210 cases and in the inpatient in 26 In the former the efflorescence was limited to the area round the lesion on (usually) the upper arm in 114 cases, but extending thence to the forearm or trunk in 86, equally often in men and women, less often in children than in adults (about 10 % against 20—25 %), and most commonly in the elderly (28 %) Bacteriological culture and attempts at virus culture of aspirated material were done in a few cases Vaccinia virus was isolated in one case, otherwise the results of these tests were negative These erysipelas like reactions initially gave rise to anxiety both in patients and doctors, since similar reactions had earlier been observed only in exceptional cases although the vaccine was the same In all cases, however, including those with the most widespread exanthema, spontaneous regression took place within the course of 2—4 days

Daughter lesions were common both in men and women, being recorded in about 10 per cent of all patients, more often in children (up to 35 %)

Lymphangitis and lymphadenitis were often seen Lymphangitis proceeding from a lesion on the extensor side of the upper arm had often a peculiar sickleshaped appearance (fig 41) Suppurating lymph glands were seen in the axilla (fig 42), but particularly in a couple of cases vaccinated on the thigh

Secondary pox, too, often gave rise to consultation with a doctor Among adults with this complication the incidence of primary vaccinees was twice as high as among other adults In the great majority of cases the secondary pox was localized to the skin, less often to superficial mucosa

Postvaccinal exanthema represented a surprisingly large proportion (20—25 %) of all complications in this series The eruptions were remarkably severe and usually urticarial in appearance (fig 43—44) the large group of Other Types in table 17 could generally be characterized most nearly as urticarial On occasions, however, the eruptions might be of virtually any type, rubella like exanthema (fig 45) was often seen, likewise exanthema of erythema multiforme exudativum type (fig 46) The exanthemas had their maximum on the vaccinated extremity but might be absent, for example, in the wristwatch area (fig 47), in other cases they were spread over the entire body

Exanthema, whatever the type but more particularly the urticarial, often had a more or less pronounced haemorrhagic element (fig 48) Among the multitude of exanthemas associated with the vaccination were also herpes zoster-like (fig 49), likewise pemphigus like vesicles and also exanthema suggestive of erythema nodosum appearing around the tenth day after vaccination Even if their occurrence might be entirely incidental, the number of such cases was nevertheless so great that they were regarded as complications to the vaccination Several cases of herpes simplex were also seen (both on skin and mucosa) a definite connection with the vaccination was easier to assume in these cases

Among the so called *malignant complications* the neurological (13 cases) pre-

TABLE 18 Interval since last vaccination in some cases of malignant complications and postvaccinal exanthema

Period since last vaccination	Neurological complications (14)	Carditis (7)	Eczema vaccinatum (7)	Postvaccinal exanthema (49)
1—3 years	–	1	–	1
4—9 years	1	–	1	3
10—20 years	1	2	1	6
Above 20 years	5	2	–	27
Not previously vaccinated	7	2	5	12
Total number of cases	*14*	*7*	*7*	*49*

TABLE 19 Heredity and history of allergy in conjunction with certain malignant complications and postvaccinal exanthema

	Neurological complications (14)	Carditis (7)	Generalized vaccinia (6)	Eczema vaccinatum (7)	Postvaccinal exanthema (49)
Heredity of allergic diseases	2	1	2	2	10
Earlier allergic manifestations	4	2	–	6	20
Allergic manifestations in recent years	1	2	–	6	3

val since the preceding vaccination was more than 10 years (Table 18) The exceptions were the cases of lymphangitis which were more numerous among those whose previous vaccination had been performed within the last 10 years than in others

Allergic heredity or history in certain malignant complications and in postvaccinal exanthema (Table 19)

Twenty of the 49 cases of postvaccinal exanthema had earlier suffered from allergic disease and 10 of them had an allergic heredity Six of the 7 cases of eczema vaccinatum had had earlier allergic manifestations or an allergic disease in the year prior to vaccination The six cases of generalized vaccinia had no history of allergy, but two of them reported allergic disease in close relatives Among the seven carditis patients two had had an allergic disease during the past year

Among the neurological complications one patient reported a heredity of contact eczema, another of other eczema Both had previously had asthma Bes-

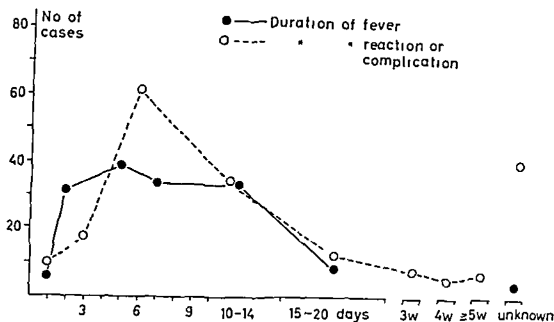


Fig 32 Duration of reaction or complication

day The majority (80 %) of the age group showing the highest incidence had been revaccinated Nevertheless the maximum for onset of reaction in the majority of cases shows a clear tendency to appear at an interval after vaccination corresponding rather to the conditions associated with primary vaccination

The fairly high incidence of reactions with onset between the tenth and fourteenth days after vaccination would appear to be due to cases of postvaccinal exanthema and to certain malignant complications Of particular interest was that the postvaccinal exanthemas generally appeared on the eighth to tenth day

A few cases of encephalitis incurred lasting invalidating sequelae which were not observed in any other kinds of complications in this series The fever was usually of a few days' shorter duration than the postvaccinal reaction A five to seven days period of fever was most common In the cases of malignant complication the fever lasted from 3—4 days up to a couple of months or more without any maximum incidence being recorded In most cases of generalized vaccinia, however, the fever disappeared within about one week

The majority of cases reacted with a maximal temperature level of above 39 5° C No fever was reported in nine cases In 36 the level was not stated

Complications in relation to interval since preceding vaccination

Of the 192 inpatients 68 had been vaccinated for the first time Among the revaccinated 90 had last been vaccinated more than 20 years previously Prior vaccination at most 20 years earlier had been done in 34 cases, less than 10 years earlier only in 16 Patients afflicted with reactions or complications necessitating hospitalization were thus predominantly persons who had had a primary vaccination or where the inter-

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nier's prurigo, other eczema or drug exanthema, but not in the past year

Thus in patients with eczema vaccinatum and postvaccinal exanthema a heredity — and to an even greater extent a history — of allergic diseases was found more frequently than in an ordinary population A heredity or history of allergic manifestations was found in about one-third of the cases of generalized vaccinia, carditis and neurological complications

Laboratory findings

Leukocytosis (> 9,000/cu mm) was observed in 24 of the 192 inpatients, in 11 of them the figure was above 12,000/cu mm A neutrophil reaction in the peripheral blood was found in 63 cases, a shift to the right in 6 The differential count showed 5—9 % eosinophils in 35 patients and more than 10 % in 11 The *thrombocytes* numbered 50,000—99,000/cu mm in 5 cases, 100,000—149,000 in 27 The *ESR* was 20—29 mm/hr in 34 patients, 30—49 in 50 and above 50 mm/hr in 30

Transient *albuminuria* was observed in 32 patients and *erythrocyturia* in 25 *Electrophoresis* revealed a rise of a_1 globulin in 3 patients, of a_2 globulin in 74, of gamma globulin in 15, hypoalbuminaemia in 13, hypogammaglobulinaemia in 11, a fully normal distribution in 45

Virus cultures in faeces were done in 30 cases, being negative in all Complement fixation tests on other virus were performed in 89 cases, a fourfold or greater rise in titre was seen in one patient only

Severity of local reactions in relation to certain laboratory findings

The local reaction to vaccination was adjudged on admission to the hospital as mild in 53 cases, moderate in 78, and severe in 54 In seven cases no classification had been made This clinical evaluation, which in all cases was done by the authors, comprised principally the appearance of the ulceration, rubor and infiltration Among the detailed records of skin status the extent of the reddened area appears for purposes of analysis to be most suited as objective measure of the severity Redness of smaller diameter than 5 cm was found in 52 cases of mild reactions, in 41 moderate and 7 severe Redness of 5—10 cm diameter was found in 1 case of mild reaction, 33 moderate and 10 severe Redness exceeding 10 cm in diameter was found in 4 cases of moderate reaction and in 37 severe Even if there is some overlap, particularly in cases with small area of redness, the evaluation should serve as a yardstick for the severity of local reactions so that they can be fairly representatively classified in the three main groups of mild, moderate and severe These have been analysed statistically in respect of certain laboratory data

The *white blood picture* showed no statistical difference between the three groups Leukocytosis and shift to the left (in a few cases to the right) were equally distributed Eosinophilia (> 5 % per 100 leukocytes) was distinctly more common in cases of severe than of mild reaction (35 % against 19 %), but the difference was not significant ($X^2 = 2.8$)

Thrombocytopenia (50 000—99,000) was found in 3 cases with moderate and 2 with severe reaction and thrombocyte be tween 100,000 and 149 000 in 4 cases with mild 12 with moderate and 10 with severe reaction, i e in altogether 4 cases of mild 27 of moderate or severe reactions (8 % against 21 %) but the difference is not significant ($X^2 = 3\ 6$)

An *erythrocyte sedimentation rate* above 30 mm was found in 76 cases, of which 15 with mild 37 with moderate and 24 with severe reaction The difference between the mild and the remaining groups is significant at the 5 % level ($X^2 = 4\ 295$ in 15/53 against 61/132 cases)

Serum protein A significant difference is observed as regards rise of α_2 globulin between mild on the one hand and moderate plus severe reactions on the other ($X^2 \sim 7\ 342$ in 12/53 against 60/132 cases) Neither zinc sulphate (flocculation) nor electrophoresis tests were done in 53 cases

A rise of γ globulin was found in 4 cases of mild 6 moderate and 4 severe reactions The zinc sulphate test (per formed in 100 cases) however showed a rise to above 15 units in 21 cases of mild reaction 40 moderate and 36 severe (the difference between the mild and severe groups is significant at the 1 % level ($X^2 = 6\ 812$ in 21/53 against 36/54 cases) Hypogammaglobulinaemia was found in 1 6 and 5 cases respectively, of mild moderate and severe reactions

A rise of *serum transaminases* was seen in 12 cases to levels between 40 and 49 in 8 cases between 50 and 74 and in 3 cases to higher levels No test was made in 41 cases all of which with mild reaction Thus slightly elevated transaminase levels were found *in some 17 % of cases* of mild reaction and some 24 % of moderate or severe reaction

The *haemagglutination inhibition test* showed elevated levels particularly in cases of moderate or severe reaction In 5 cases of mild, 15 of moderate and 17 of severe reaction there was a more than fourfold rise of titre between serum from the time of hospitalization and the convalescent phase the difference between cases of mild and of moderate or severe reaction was significant at the 5 % level ($X^2 = 4\ 572$ in 5/53 against 32/132 cases) Complement fixation tests showed no significant differences between the groups

Serovirological results correlated to certain complications and laboratory findings

The maximum haemagglutination inhibition (HI) titres were below 5 in 35 cases 20 in 57 40—160 in 60, and above 160 in 7 cases In 23 patients the titre was not determined or no result was recorded in 47 cases the titre was determined only once No rise or a rise of titre between acute phase and convalescent phase serum of less than fourfold magnitude was recorded in 87 cases a fourfold rise in 19 and more than fourfold in 39 The complement fixation test showed a maximum of below 5 units in 22 cases 5—20 in 81 40—160 in 43 and above 160 in 3 No test was made in 43 cases No rise or a less than fourfold rise of titre between acute phase and convalescent phase serum was recorded in 79 cases, a fourfold rise in 29 more than fourfold in 30 Data are lacking in 34 cases

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zema vaccinatum and gangrenous vaccinia were given 1 ml per kg body weight of vaccinia immune gamma globulin daily, the worst afflicted also thiosemicarbazone In some patients not given these treatments the symptoms regressed as quickly or as slowly as in the treated The series are too small to allow definite conclusions

Discussion

On a rough approximation it may be said that 300 000—350 000 persons were vaccinated in central Stockholm As a general rule patients with severe reaction or complication will have been referred by their physicians to the hospital from which this report is made We take it therefore that our material contains the majority of postvaccinal reactions that have given cause for anxiety By a further approximation we get an incidence of 1 300 cases in which vaccination demanded medical consultation and care and in round figures 1 1 000—2 000 vaccinees requiring hospitalization The time in hospital was sometimes 1—2 weeks more often only a few days

The vaccine used had been employed for several years (16 18) and was stronger than that used 10—20 years ago In this mass vaccination the large number of serious reactions was nevertheless surprising and there were those who wished to attribute it to the vaccine This has manifestly been the case in several earlier mass vaccinations (2) But when later evaluating the incidence of reactions and complications — with the approximations that have been made — this mass vaccination does not appear to have caused a greater number of complications than in earlier epidemics — rather, in fact, less (23, 26) Not a single death occurred as a result of vaccination, and this is perhaps our chief experience

Judging from the literature (24) the incidence of postvaccinal exanthemas in this material does nevertheless appear to be especially high, even if these complications may have been more common in times past than in recent years (20) The exanthemas were often of allergic type and were originally thought to be due to the vaccine being based on an egg medium The allergological tests revealed no such relation, however, nor were allergenic components found in other fractions of the vaccine (46)

In this material however, the cases of postvaccinal exanthema had a heredity and history of allergic diseases to a greater extent than might be expected in an ordinary population The same applied, of course to the patients with eczema vaccinatum Whether allergic elements may be a factor also in certain other reactions and complications is open to question (12 13 44 45) That this may be so is suggested by the fact that these reactions and complications, like the postvaccinal exanthemas usually had their onset one week after the vaccination and the administration of the foreign antigen On several previous occasions the possibility of a tissue hyperergic reaction phase after the vaccination has been discussed (13) especially in conjunction with the postvaccinal exanthemas (12) and generally in the form of delayed hypersensitivity (19, 43 a,

The HI titres were not higher in cases with than without *eosinophilia* if solely the highest HI titre measured in each patient is considered Eosinophilia occurred more often, on the other hand, in cases with fourfold or higher rise of titre between onset and convalescence than in cases with lower or no rise of titre (42 % against 17 %), the difference being significant at the 1 % level ($X^2 = 9\ 162$ in 23/38 against 32/104 cases)

Elevated *a_2-globulin* was seen in 38 % of the cases with no or less than fourfold rise of titre but in 60 % of those with at least fourfold rise, the difference is significant at the 1 % level ($X^2 = 6\ 14$ in 33/87 against 35/58 cases)

No difference correlated to titre or rise of titre was recorded in other laboratory tests (white blood cells, haemoglobin, thrombocytes, ESR, serum transaminase, gamma globulin)

Culture assays for isolation of virus from tissue aspiration around the lesion were made in a few cases (17) They were positive in five patients whose rise of titre was less than fourfold and in seven with more than fourfold rise

Lymphangitis and/or evident lymphadenitis was found in 2 % of patients with less than fourfold and in 10 % with at least fourfold rise of titre Secondary pox in the eyes and in the anogenital region was likewise seen more often in cases with fourfold or greater rise of titre than in cases without such serological reaction — twice or thrice as often — but the differences were not significant

No differences were found between patients with and without a pronounced rise or high level of titre as regards other complications such as postvaccinal exanthema, vaccination encephalitis, myocarditis

Corresponding calculations as regards level and rise of titre of *complement fixing antibodies* showed similar results in respect of white blood picture, ESR, a_2-globulin and postvaccinal complications, but in no instance with statistically significant difference A rise of a globulin was found in 36 % of cases with titre less than 5 and in 48 % with higher titre No cases of encephalitis, anogenital secondary pox or myocarditis had a titre less than 5, but among encephalitis cases a fourfold or higher rise in titre was found in 4, and among cases of anogenital secondary pox in 6 cases Regional adenitis was about four times more common in cases with at least fourfold rise of titre than in other patients

Therapy

Cases with pronounced, apparently purulent local affections were initially treated with antibiotics, but we abandoned this therapy after bacteriological cultures had shown negative results in a large number of cases We saw no difference as regards duration of fever or regression of local lesions between patients who had and had not been given systemic or local antibiotic treatment Severe suppurating lesions were washed daily with potassium permanganate solution Otherwise the time honoured principle was followed of simply covering the lesion with a loose and airy bandage

During the first days the most serious cases of neurological complication ec

local reaction varies in direct proportion to the quantity of antigen administered (18 43a) In the present material, however the quantity of antigen can hardly have varied very much from one case to another and we consider it most probable that differences in strength of local reaction were due mostly to individual variations in hypersensitivity in the same way as in the "malignant complications

In animal experiments one has found no relation between the delayed hypersensitivity reaction to vaccinia and the quantity of circulating antibody The spread of vaccinia virus in the skin was considered to be normally limited by factors other than antibody and delayed hypersensitivity (50) The interferon mechanism probably has a significant role

From the start our attention was directed to the possibility of complications deriving from many organs and organic systems for example bone involvement (7 14) or in the form of keratitis (51) myelitis (40) or nephritis (21, 30) Several cases admittedly had erythrocyturia and albuminuria but these phenomenons all were quickly transient and in no case gave rise to deterioration of renal function Nor was thrombocytopenic purpura seen among the complication occasionally described as rarities (41) but the fairly numerous cases of thrombocytopenia suggest that this is a complication which cannot be disregarded Our series also shows that a rise of serum transaminase was usual and an elevated zinc sulphate value was not seldom seen, which suggests that the liver function may be affected

As regards treatment new methods were tried only by thiosemicarbazone (3, 4, 5, 28, 49) Successful results have earlier been reported with vaccinia-immune gamma globulin (10, 33, 34, 35, 36, 43) This drug was given to the majority of definite and suspect cases of encephalopathy and to cases of eczema vaccinatum Thiosemicarbazone was given to a few of these cases Our series is too small for any conclusions to be drawn from it Marked clinical improvements, however, were seen in some cases from one day to the next even before any of the agents had been administered In others a grave picture of encephalopathy remained unchanged after administration of both drugs It is obviously difficult to make adequate arrangements for representative series to illustrate the efficacy of these agents Our experience gave little encouragement Progressive and gangrenous vaccinia in agammaglobulinaemia has in other investigations not been improved by gamma globulin or vaccinia immune gamma globulin, which was thought to be due not only to an inability to form certain antibodies but also to defective tissue immunity (12) Dissociation between gamma globulin production and antibody formation has also been observed (27) Other authors have found smallpox vaccination to run a smooth course despite antibody deficiency syndrome (agammaglobulinaemia) (29 32) Interferon which has been tried by other authors was not used by us

During the years 1924—1936 there were 54 cases of vaccination encephalitis in Sweden after more than 1 1/2 million vaccinations (mostly primary vac

44, 50) Complications such as urticaria and generalized vaccinia also appear to be more common in persons with skin diseases (8, 42)

Mild eosinophilia was found in 25 per cent of the in patients, but not significantly more often in cases with, for example, postvaccinal exanthema Eosinophilia cases, on the other hand, had fourfold rises of HI titre significantly more often than cases without eosinophilia This should lend indirect support to the view that the pathogenesis of postvaccinal complications includes a hypersensitivity factor — even if rises of HI titre could not be directly correlated to the occurrence of such complications

Significant differences between different sections of the material were found for the HI reactions, but not for CF titres This difference between the two reactions is clearly evident from a comparison between the titres of smallpox patients, of cases of postvaccinal complication and of the normal population, which is presented in another part of this supplement (17)

Patients vaccinated despite a chronic myeloic leukaemia or in the course of radiotherapy or steroid therapy have in several cases had a prolonged generalized vaccinia or gangrenous vaccinia On the other hand it has been found that irradiated animals in which no neutralizing antibodies against vaccinia could be detected recovered from vaccinia infection as rapidly as non irradiated animals, suggesting that the production of neutralizing antibodies was not necessary for recovery (22) This appears to suggest that vaccination allergies do not require humoral vaccinia antibodies but are chiefly cell bound allergies (12)

Elevation of HI titre, as of ESR and α_2-globulin, has been found to be a significant measure of the strength of local reactions Patients with severe reactions in this material also had eosinophilia and thrombocytopenia to a greater extent than those with weaker reactions Elevated ESR and α globulin reflect the strength of the acute inflammatory reaction and possibly also of disintegration of tissue The thrombocytopenia may be compared with that fairly regularly found in smallpox and sometimes, too, in other infections It was directly related to the strength of the local reaction but undoubtedly also to the haemorrhagic character of certain postvaccinal exanthemas The serological antibody response was more pronounced in cases with than without complications (17) When the size of the material permitted a statistical analysis, significantly greater rises of titre were found in cases with strong than with weaker reactions The local vaccination reaction thus leads to a severe acute inflammatory reaction (α globulin), thrombocytopenia and some eosinophilia, and also to a marked antibody response The absence of leukocytosis, shift to the left, and the usually negative response in bacteriological cultures show that the inflammatory reaction around the inoculated area seldom has a bacteriological aetiology (25) With some exceptions the local reaction would appear to be caused by vaccinia virus (43 a) In a single case in this investigation this virus was isolated in tissue aspiration drawn more than 10 cm from the lesion The strength of the

local reaction varies in direct proportion to the quantity of antigen administered (18, 43a) In the present material, however, the quantity of antigen can hardly have varied very much from one case to another and we consider it mo t probable that differences in strength of local reaction were due mostly to individual variations in hypersensitivity in the same way as in the "malignant complications

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During the years 1924—1936 there were 54 cases of vaccination encephalitis in Sweden after more than 1 1/2 million vaccinations (mostly primary vac

cinations), i e 1 29,000 (47), rather higher than in other reports but of roughly the same order as in the present instance Ten of these cases were fatal During the same period there were 14 cases of smallpox, one of which was fatal From 1948 to 1957 there were reports of 25 cases of vaccination encephalitis in Sweden, chiefly at pre-school age Two of them died, and no case of smallpox was reported in that period (15) Against a background of similar assumptions an editorial in The Lancet brought up the question of vaccination for renewed consideration (48) Experience from this Swedish mass vaccination shows extremely clearly that the severe reactions and complications which occur even after an individually more or less carefully performed vaccination afflict the primary vaccinees and those vaccinated a long time previously On the other hand the benign reactions, although often intense, were always quickly transient The malignant complications, principally the neurological, led to invalidating sequelae only in a few cases and in no case to death We are somewhat doubtful about the effect and the freedom from risk of the new therapeutic agents that have appeared in recent years, and our presumption is that revaccination, e g every tenth year, so substantially diminishes the incidence of serious postvaccinal complications that this is preferable to primary vaccination in adulthood on account of a threatening epidemic or a planned journey abroad The vital point, of course, is the difficulty of organizing voluntary vaccination of the entire population at 10 year intervals

Summary

In conjunction with voluntary mass vaccination of approximately 350,000 persons in central Stockholm against smallpox, the inpatient and outpatient care of postvaccinal complications was centralized at the Hospital for Infectious Diseases 884 persons were accepted as outpatients, 192 as inpatients Among the former roughly every fourth person presented either for erysipelas-like efflorescences, lymphangitis and lymphadenitis or postvaccinal exanthema Among the latter 7 % were treated for neurological complications, 3—4 % each for myopericarditis, eczema vaccinatum or benign generalized vaccinia A few cases had suspect gangrenous vaccinia

The majority of patients were women aged 15—60, and by far the greatest number of complications or severe reactions were in primary vaccinees or persons who had last been vaccinated more than 10—20 years ago

The postvaccinal reactions usually started on the 7th day and lasted in a couple of cases up to 10—14 days (in some cases considerably longer)

Cases with severe local reactions and with postvaccinal exanthema were both numerous Eosinophilia, thrombocytopenia, hypogammaglobulinaemia and elevated transaminases, also (and with statistically significant differences) elevated ESR, α-globulin zinc sulphate flocculation and haemagglutination inhibition titre, were observed more frequently in cases of severe than of mild local reactions An elevated titre or fourfold or greater rise of haemagglutination titre was seen especially in cases of

lymphangitis lymphadenitis and secondary pox and at a statistically significant level in cases of eosinophilia and elevated α_2 globulin

Thus there appears to be a hypersensitivity factor in the pathogenesis of cases with severe local reactions Heredity and earlier history of allergic diseases were common in cases of postvaccinal exanthema and eczema vaccinatum but relatively common also in other malignant complications so that allergic factors may be thought to have an aetiological role also in the latter

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Neurological Complications after Smallpox Vaccination

Borje Holmgren and Ulf Lindblom[1]

During the smallpox outbreak fourteen patients with neurological complications following smallpox vaccination were admitted to the Stockholm Hospital for Infectious Diseases between May 25 and July 19, 1963 Ten of them had cerebral signs and symptoms with or without elevation of the cell count in cerebrospinal fluid, three had solely meningitic signs, and one an isolated sixth nerve paralysis The cerebral symptoms are customarily denoted as encephalitis but in this paper we prefer the term *encephalopathy* This is more correct since the collected data now at our disposal provide a strong indication that the underlying process is of primarily demyelinizing or toxic type (13 12) and not a direct attack of the virus on the nervous tissue as in genuine encephalitides such as RSSE polio encephalitis and the equine encephalitides

Clinical data

The most important data from the 13 cases with cerebral and/or meningeal complications are summarized in Table 20 Only 5 were males which may be due to chance or to the sex distribution of the vaccinated persons which however is unknown All ages are represented only 3 being children This age distribution probably reflects a predominance of adults among the vaccinated persons, as well as the earlier known fact that the risk of encephalopathy increases with age (6 7)

In as many as seven of the cases the complication arose after revaccination, which is remarkable considering that the incidence of encephalopathy earlier has been significantly higher after the *first vaccination* (6 and others) but in the present cases a long time had elapsed between the first and subsequent vaccinations (on an average 40 years) It is obvious that so late a revaccination is immunologically comparable to primary vaccination Unfortunately we do not know the distribution of primary and secondary vaccinations during the period in question

The incubation periods were typical the shortest 7 and the longest 13 days In one case (no 2) the period cannot be stated more exactly than that it was less than 18 days This was an unvaccinated eczema patient who lived with his newly vaccinated mother and was infected from her on some occasion

The symptomatology accords well with the earlier known picture of postvaccinal encephalopathy and meningitis The signs and symptoms have been

[1] Dept of Neurology Karolinska Sjukhuset Stockholm Sweden.

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Symptoms						Cerebrospinal fluid			EEG				
Nuchal rigidity	Lasègue pos	Headache	Vertigo	Nausea	Photophobia	Pressure mm H_2O	Cells/mm³	Total protein mg %	1 week	2 week	3 week	4 week	>4 week
⊗		●		○		135	788	115	●	⊗	⊗	○	?
●		●		○			9	44					○
●	○	○					184	64	●	○	?	N	N
○			○				2	32	○	?			N
⊗	○			○		1/0	2	170	⊗	⊗	○	○	?
○				○		100	3	68	⊗	⊗			N
⊗		●				100	24	5[illegible]	\	○		N	\
○		●		○		105	160	26	⊗	○		?	N
		⊗	○	○			2	3[illegible]	⊗		○		\
		⊗	○		○		5	79	N		N		
	○	○	○	○		130	106	40	N		\	\	
		○		⊗			60	78		○			\
○	○	○	○		○		20	32	N	\			

cerebral signs which occurred after the usual incubation period and ran a typical course (The subjective symp toms could in no case be regarded as specifically cerebral) In the three mild cases the diagnosis was based on the symptoms together with the laboratory findings (see below)

In cases 11—13 the symptoms were compatible with meningitis as much as with encephalopathy Apart from nuchal rigidity *in one of them (no 13) the* only objective signs were elevated cell counts in cerebrospinal fluid and they were therefore classified as purely me ningeal complications In case 12 the electroencephalogram (EEG) was slight ly pathological in the second week. This finding alone was not considered as evidence of encephalopathy Unfortu nately no EEG was recorded in the first week.

The fourteenth case a 2 year old girl (record no 1857/63) was assessed a postvaccinal *mononeuropathy* On the fifth day after primary vaccination an acute right sided paralysis of the sixth nerve *set in which successively receded within* the course of 3 months The clinical ex amination gave no indication of en cephalopathy The EEG showed nothing abnormal Haemorrhage occured on

Table 20

Record no 1963	Case no	Sex	Age	Prim vaccination	Years since last vacc	Interval, days (incub)	Signs: Loss of consciousness	Disorientation	Hallucination	Slow cerebration	General rigidity	Babinski's sign	Convulsions
1879	1	♀	49		45	9		●				⊗	
2008	2	♂	20	+		<18	⊗	●	⊗		O		
1944	3	♂	6	+		11	⊗				⊗	⊗	O
1960	4	♂	58		55	11		●					
2003	5	♂	10	+		13				⊗			
1955	6	♀	73		70	11		⊗					
1953	7	♀	56		50	11		⊗					
1816	8	♀	25		6	8							
1936	9	♀	11	+		9							
1933	10	♀	45	+		7							
1825	11	♀	18		15	7							
2334	12	♀	25	+		12							
2052	13	♀	40		35	11							

N = *normal* ? = *borderline* ● = *high*, ⊗ = *moderate* O = *low intensity*

graded according to their intensity In Table 20 filled circles denote high, crossed circles moderate and empty circles low intensity *Only definite findings* are included among signs in the table, doubtful ones being excluded Cases 2 and 3 suffered from unconsciousness of moderate degree (sopor) Three patients were seriously disorientated, one of them also had *hallucinations* (case 2) The youngest patient (no 3) had mild convulsions and elevated tonus of the rigidity type Besides this case, convulsions were not observed, probably because the material included so few children Most cases displayed stiffness of the neck

The table also shows the incidence of subjective symptoms Headache and nausea, as expected, were most common *Headache* was absent in *three* cases Two of them (cases 4 and 6) may have had headache while disorientated Symptoms are included in the table only i they were pronounced and lasted for several days

The ten *encephalopathic* cases are ranked in the table by degree of severity Cases 1—4 are classified as serious, cases 5—7 as moderate and cases 8—10 as mild In cases 1—7 the diagnosis of postvaccinal encephalopathy was assured by *the observation of definite*

Symptoms						Cerebrospinal fluid			EEG				
Nuchal rigidity	Lasègue pos	Headache	Vertigo	Nausea	Photophobia	Pressure mm H_2O	Cells/mm³	Total protein mg %	1 week	2 week	3 week	4 week	> 4 week
⊗		●		○		135	788	115	●	⊗	⊗	○	?
●		●		○			9	44					○
●	○	○					184	64	●	⊗	?	N	N
○			○				2	32	⊗	?			N
⊗	○			○		170	2	170	⊗	⊗	⊗	○	?
○				○		100	3	68	⊗	⊗			N
⊗		●				100	24	55	N	○		N	N
○		●		○		105	160	26	⊘	○		?	N
		⊗	○	○			2	35	⊗		○		N
		⊘	○		○		5	79	N		N		
	○	⊘	○	○		130	106	40	N		N	N	
		○		⊗			60	78		○			N
○	○	○	○		○		20	32	N	N			

cerebral signs which occurred after the usual incubation period and ran a typical course (The subjective symptoms could in no case be regarded as specifically cerebral) In the three mild cases the diagnosis was based on the symptoms together with the laboratory findings (see below)

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The *fourteenth case* a 2 year-old girl (record no 1857/63) was assessed a postvaccinal *mononeuropathy* On the fifth day after primary vaccination an acute right sided paralysis of the sixth nerve set in, which successively *receded within* the course of 3 months The clinical examination gave no indication of *encephalopathy* The EEG showed nothing abnormal Haemorrhage *occured* on

lumbar puncture, and mild changes of the cerebrospinal fluid which were concealed by the haemorrhage cannot be excluded The reasons for considering this case as a postvaccinal complication will be given below (p 111)

Occasional cases of postvaccinal radiculitis are described in the literature (10 and others) There may have been a mild polyneuropathy in two cases observed by us One was a 40-year-old woman (record no 1845/63) with glove-type paraesthesiae in the hands and lower arms lasting from the 14th to the 21st day after vaccination There was no paralysis or reflex disorder, and the cerebrospinal fluid protein was normal The other was the encephalopathy case no 5, in which the deep reflexes were weakened and the cerebrospinal fluid protein elevated in the acute stage, but without paralysis or disturbance of sensibility The weakening of the reflexes, however, might have been due to diminished tone on account of the encephalopathy The rise of protein may also have been secondary to the cerebral lesion

Laboratory findings

The *cerebrospinal fluid* findings in the encephalopathy cases varied from fully normal (cases 4 and 9) to a moderate rise of the cell count alone (no 8), of the protein alone (nos 5, 6 and 10), or of both (nos 1, 3 and 7) It is remarkable that the pressure was normal in all cases in which it was measured

In case 10 the diagnosis of encephalopathy was based on the symptoms together with the elevation of the protein in the cerebrospinal fluid We considered it most likely that the rise of protein was secondary to cerebral lesions Two alternative interpretations are theoretically possible There may have been a laboratory error, or the rise may have been due to radiculitis But there were no indications of a laboratory error nor clinical signs or symptoms of radiculitis

Electroencephalography (EEG) was carried out as a rule once a week during the acute phase and at least once during the follow up period In most cases there was a generalized abnormality, which receded in parallel with or rather more slowly than the clinical signs and symptoms The abnormality was most pronounced in the serious cases In the table, filled circles imply that the normal EEG activity was absent and replaced by slow waves of high amplitude Crossed rings signify moderate abnormality, i e a tracing with elements of normal activity mixed with slow waves, and empty rings slight abnormality In the latter case the basic rhythm was normal and the pathological activity sporadic In the normalization phase some tracings were probably but not definitely pathological These are indicated by a question mark in the table

As will be seen from the table, with two exceptions (nos 2 and 7) the electroencephalograms were similar in all serious and moderate cases of encephalopathy, i e the cases in which the diagnosis was established on the basis of clinical cerebral signs In case 2 no EEG could be recorded during the first month on account of the poor condition of the patient In case 7 the EEG was pathological only in the second week,

when the clinical signs and symptoms had already started to recede We have no explanation of this deviation

Cases 8 and 9 displayed EEG abnormalities of the same type as in those with definite clinical cerebral signs, and here we took the EEG finding as an evidence of encephalopathy

Virology It is well known that post vaccinal encephalopathy has occurred epidemically in other countries, for example Holland and Britain This gave rise to speculations at an early stage as to whether another virus might be involved in the pathogenesis of the cerebral lesions (9 and others) During the present investigation particular interest was devoted to this question by examining the blood serum for the presence of complement fixing antibodies to polio RSSE mumps measles and herpes simplex In no case was there any significant shift of titre between acute and convalescent phase sera Virus cultivation from faeces and/or cerebrospinal fluid was made in ten of the cases likewise with negative results It may be added that our cases displayed no epidemic characteristic but were evenly distributed throughout Stockholm During the period under consideration there was a limited epidemic of Echo 9 in one of the western suburbs Bromma) None of the cases of postvaccinal encephalopathy were from that area

Sequelae

None of the patients died but three displayed sequelae In case 1 the encephalopathy was followed by a psychotic illness for which in April 1964 she was still being treated in a mental hospital in the ward for severely disturbed patients Case 2 developed a psychasthenic syndrom which has hitherto rendered rehabilitation impossible Case 10 also showed a psychasthenic insufficiency state which slowly improved but for which she was still on the sick list in April 1964 None of the patients with sequelae had manifested any similar symptoms before the vaccination All had been healthy and fully capable of work

Discussion

No definite figures can be given of the incidence of neurological complications in connection with the smallpox vaccination campaign since the number of vaccinated persons is unknown On a rough estimate at least half a million people were vaccinated within the entire Stockholm area Besides the 14 cases described here six cases of encephalopathy were admitted to the Stockholm County Hospital (Danderyd) This would imply 20 neurological complications to at least 500 000 or 1 to at least 25 000 vaccinations

Seven of our cases were taken ill after late revaccination It seems advisable to avoid too long intervals between primo and revaccinations Routine revaccination of women for example on leaving school or at the age of 20, would be appropriate Men are usually revaccinated in conjunction with their military service Alternatively one might consider giving immunoglobulin on late revaccination for a recent Dutch investigation clearly showed that im

munoglobin can lower the incidence of encephalopathy (8) An increased routine vaccination would bring a certain annual increase of postvaccinal complications in the country, including, at an estimate, one or two cases of encephalopathy On the other hand one might hope for a reduction of complications following mass vaccinations performed during epidemics

Postvaccinal encephalopathy is the most serious complication following smallpox vaccination The mortality has earlier been relatively high (11) In Sweden it was on an average 20 per cent (10 of 54 cases) in the years 1924—36 (6) For the survivors the prognosis is good, a quick and more or less complete recovery is characteristic even if grave neurological signs and symptoms have been recorded in the acute phase Residual signs occur, however, (5), which shows that there may be a permanent anatomical injury Two of our serious cases had sequelae which may be due to persistent cerebral lesions Alternatively, in case 1, in which *psychosis followed, the postvaccinal en*cephalopathy may have had only a triggering role Case 2 exhibited exogenous elements which may have contributed to the mental insufficiency His hair had fallen off and his face was deformed by many pock marks In our third case (no 10) the encephalopathy was mild in the acute phase and a permanent anatomical lesion is therefore less likely

It is well known that the severity of the encephalopathy varies from case to case Probably in a large material all degrees of severity will be represented This variation indicates that mild cases may occur which do not exhibit sufficiently manifest symptoms to permit the diagnosis During the period of this study eight patients were admitted to the Stockholm Hospital for Infectious Diseases with pronounced headache and/or dizziness which persisted after the local vaccination reaction had started to disappear There was no reasonable explanation in their histories such as for example, a tendency to headache, dizziness or psychosomatic symptoms All tests, including LP and EEG, were negative The possibility of minor encephalopathic changes in these cases cannot be excluded It should be pointed out, however, that even if such subclinical encephalopathies exist, there are no grounds to suggest that any permanent anatomical changes should follow The good recovery of the severe cases indicates instead that initially mild cases heal completely This is important to bear in mind in the treatment of patients with a tendency to neurotic fixation to a supposed organic cerebral lesion

The EEG findings do not differ from *those found in other forms* of diffuse or multifocal acute cerebral disorders such as the postexanthematous encephalopathies and viral encephalitides (4, 3) Although the electroencephalographic changes are not specific, the examination may be a diagnostic aid To be so, repeated electroencephalograms should be recorded in the acute phase so that the course can be followed and compared with the clinical one

According to current neuropathological opinion one cannot decide on the pathoanatomical picture of encephalop-

athy from the clinical symptoms or vice versa (12) The so called classical picture of perivascular demyelinization is by no means constantly present As an example, it did not occur in any of the four lethal cases clinically diagnosed as postvaccinial encephalopathy following the mass vaccination in New York in 1947 (2, 1) We have no evidence as to whether primary demyelinization or less specific toxic lesions were present in our cases except possibly for case no 2 This patient had a malignant eczema vaccinatum and was severely ill with a prolonged fever of above 40° C A disturbance of the electrolyte balance or a toxic effect of the extensive skin lesions may have provoked or contributed to the cerebral signs in this case

Spillane and Well (1964) made a distinction of encephalomyelitis and encephalopathy on the basis of clinical signs and symptoms and supposed histopathological picture We have not found such a classification possible There was no further differentiation of the clinical picture within the group of patients with cerebral signs

Cranial nerve paralysis may appear, after smallpox vaccination as an element in a general picture of encephalopathy (for example Scott's cases nos 9 11 and 12) Peripheral nerve injury is however uncommon (14) and isolated cranial nerve paralysis would seem to be an extreme rarity A coincidence between the vaccination and the sixth nerve paralysis may therefore be a possibility in our case no 14

That an independent disease may occur in a deceptive manner is illustrated by the following case A 50-year-old women who had been revaccinated on account of the epidemic fell ill on the fourteenth day after vaccination with a partial left sided paralysis of the third nerve and was admitted to the hospital on a suspicion of postvaccinal neuropathy Carotid angiography, however, revealed a hazel nut sized aneurysm on the base of the brain which was undoubtedly the cause of the paralysis However in the case of sixth nerve paralysis no other plausible reason for the paralysis was found and the case was therefore taken to be a post vaccinial neuropathy

Summary

Neurological complications after smallpox vaccination During the smallpox outbreak in Stockholm approximately 500 000 people were vaccinated within the course of a few weeks in the entire Stockholm area Of the cases with post vaccinal neurological complications 20 were treated in hospitals Fourteen of these were treated at the Stockholm Hospital for Infectious Diseases and are described in this paper 5 were males and 9 females, all ages be represented In 7 cases the complication arose after revaccination in most of these cases several years had elapsed after primary vaccination 10 cases had cerebral encephalopathy 4 being classified as severe with respect to the cerebral signs 3 as moderate and 3 as mild 5 of these 10 cases also had increased cell count in cerebrospinal fluid Another 3 of the 14 cases had only a mild meningeal reaction and 1 had a mononeuropathy There was no fatal outcome but 2 of the cases

with severe encephalopathy and 1 of those with moderate encephalopathy had longlasting psychic symptoms

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From The Hospital for Infectious Diseases Stockholm

Circulatory Studies in Patients with Abnormal ECG in the Course of Postvaccinal Complications

ELIAS BENGTSSON ALF HOLMGREN and BERTIL NYSTROM

Myocardial and/or pericardial complications after smallpox vaccination have been reported to occur 1—3 weeks after the inoculation (7 10 13 14 21 22) primarily in adults but also in children (7 10 12 18) Whether they are due to the viraemia that accompanies the inoculation or are caused by a hypersensitivity reaction has been discussed (2 7) These types of complication (myopericardial reactions) have been repeatedly verified at autopsy they consist of interstitial infiltrations of predominantly round cells and have been regarded as lethal in exceptional cases (10)

The present investigation was undertaken to study the frequency of electrocardiographic changes in a group of patients hospitalized because of other complications after smallpox vaccination The study forms part of a survey of postvaccinal complications in conjunction with the Stockholm smallpox epidemic in 1963

Patients with abnormal ECG were subjected to measurements of blood volume heart volume and exercise tolerance in an attempt to evaluate the circulatory significance of the myocardial lesion

Material

The material consists of 18/192 cases, hospitalized at the Stockholm Hospital for Infectious Diseases because of other complications (6) after the volontary mass vaccination that took place during the 1963 smallpox outbreak All patients had an ECG taken on admission Patients with normal ECG (13/18 cases) and patients with symptoms and signs as precordial or retrosternal pain, dyspnoea, oppression cardiac arrythmia tachycardia or palpitations were selected for the present study No patient had any signs indicative of heart insufficiency

During the period in hospital the patients were classified on the basis of the severity of the ECG changes into two groups group I with marked ECG changes (7 cases) and group II with moderate changes (6 cases) Five other patients were included in group II who had normal ECG but symptoms of the same type as the rest of the material

Group I consisted only of men, varying in age between 20—66 years (mean 33 years) None had heart disease before the vaccination Group II con

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TABLE 21 Collected clinical and laboratory datas

Patient no	Sex	Age	Diagnosis[1]	Previous cardiovascular and related diseases	Symptoms: Days for onset after vaccination	Precordial or retrosternal pain	Shortness of breath	Oppression	Palpitation	Other complains
I 1	♂	66	M	0	10	+	+	–	–	
I 2	♂	25	MP	0	14–21	–		+		+
I 3	♂	39	M	0	?	+	+	+	–	–
I 4	♂	20	M+Ecz vacc	0	–	–	–		–	
I 5	♂	21	MP+Pharyngitis +Furunculosis	0	8	+	+	–	+	–
I 6	♂	39	M+P? + Erysipelas	0	9	–	–	+	–	–
I 7	♂	20	M+Ecz vacc septichemia?	0	?	?	?	?	?	?
II 1	♀	25	Encephalitis	0	–		–	–	–	–
II 6	♀	12	Ecz vaccinat	0	–	–	–	–		–
II 4	♀	58	—	Thyreotoxicosis	–	–	–			–
II 5	♀	45	Postvacc exanth	Vasoregulatory asthenia	–		–	–	–	–
II 2	♂	35	Postvacc exanth	0		–		–	–	–
II 3	♂	45	Bronchitis	0	9	+	–	–		+
II 7	♀	60	—	Stenocardia	7	–	+	+	–	–
II 8	♀	52	—	0	?	–	+		–	–
II 9	♀	35		0	6	+	+	+	+	+
II 10	♀	72	Lymphangitis	Hypertension	12	–	+	–	–	–
II 11	o	18	—	0	6	–		–	–	+

[1] M—myocarditis P—suggestive pericarditis

[2] Less equal or more than fourfold between different examinations

[3] Maximal recorded value

sisted of three men and eight women, 12—72 years (mean 45 7 years) of age

Three patients in group II (cases 5, 7 and 10) had earlier suffered from heart disease Case II 7 (60 years of age) had

Culture from		HI Titre		CF Titre			Serum transaminases[3]		
Pox	Nasopharyngx	Maximal	Rise[1]	Maximal titre	Rise[1]	Leucocytes[3]	SGOT	SGPT	ESR/1 h[3]
—	—	160	4	160	4	10 500	26	27	36
β h streptoc	Staph aur	20	—	40	>4	12 400	36	24	24
—	—	—	—	80	—	8 000	63	69	12
Staph aur	Staph aur	1 280	<4	640	—	11,200	33	45	7
Staph aur	Pneumococc	40	<4	—	—	8 200	28	20	18
—	Staph aur	320	<4	—	—	6 000	—	—	42
Coli + Staph aur	β h. streptoc. + Staph aur	640	>4	16	—	16 800	24	19	115
—	—	160	4	—	—	6 600	17	14	22
	—	10	4	—	—	6,200	—	—	50
—	—	320	>4	—	—	7 200	25	35	45
—	—	160	4	—	—	7 000	17	16	8
	—	<5	<4	—	—	6,500	—	—	44
—	—	20	<4	—	—	6 800	28	25	48
	—	80	>4	—	—	6 600	—	—	42
	—	40	<4	—	—	5,200	—	—	16
	—	20	<4	—	—	4 000	—	—	32
	—	—	—	—	—	5 400	45	20	36
	Pneumococc	—	—	—	—	3,500	28	24	4

Group I represents seven patients with marked ECG changes Group II 1—II 6 represent patients with moderate ECG changes and group II 7—II 11 patients with normal ECG

diphtheria in 1918 and had suffered from precordial pain and palpitations in the last few years Case II 5 (45 years) had neurotic heart symptoms in 1961 Case II 10 (72 years) had arterial hypertension

TABLE 21 Collected clinical and laboratory datas

Patient no	Sex	Age	Diagnosis[1]	Previous cardiovascular and related diseases	Symptoms					
					Days for onset after vaccination	Precordial or retrosternal pain	Shortness of breath	Oppression	Palpitation	Other complains
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I 2	♂	25	MP	0	14–21	–	–	+	–	+
I 3	♂	39	M	0	?	+	+	+	–	–
I 4	o	20	M+Ecz vacc	0	–	–	–	–	–	–
I 5	♂	21	MP+Pharyngitis +Furunculosis	0	8	+	+	+	+	–
I 6	♂	39	M+P? + Erysipelas	0	9	–		+	–	–
I 7	♂	20	M+Ecz vacc septichemia?	0	?	?	?	?	?	?
II 1	♀	25	Encephalitis	0	–		–	–		–
II 6	♀	12	Ecz vaccinat	0	–		–	–	–	–
II 4	♀	58	—	Thyreotoxicosis	–	–	–	–	–	–
II 5	♀	45	Postvacc exanth	Vasoregulatory asthenia	–		–	–		–
II 2	♂	35	Postvacc exanth	0		–	–	–		–
II 3	♂	45	Bronchitis	0	9	+	–			+
II 7	♀	60	—	Stenocardia	7		+	–	–	–
II 8	♀	52	—	0	?	–	+	–	–	–
II 9	♀	35	—	0	6	+	+	+	+	+
II 10	♀	72	Lymphangitis	Hypertension	12	–	+	–		–
II 11	♂	18	—	0	6	–	–	–		+

[1] M – myocarditis P – suggestive pericarditis

[2] Less equal or more than fourfold between different examinations

[3] Maximal recorded value

sisted of three men and eight women, 12—72 years (mean 45 7 years) of age

Three patients in group II (cases 5, 7 and 10) had earlier suffered from heart disease Case II 7 (60 years of age) had

Culture from		HI Titre		CF Titre			Serum transaminases[4]		
Pox	Nasopharyngx	Maximal	Rise[2]	Maximal titre	Rise[2]	Leucocytes[3]	SGOT	SGPT	ESR/1 h[4]
—	—	160	4	160	4	10 500	26	27	36
β h streptoc.	Staph aur	20	—	40	>4	12 400	36	24	24
—	—	—	—	80	—	8 000	63	69	12
Staph aur	Staph aur	1 280	<4	640	—	11,200	33	45	7
Staph aur	Pneumococc.	40	<4	—	—	8 200	28	20	18
—	Staph aur	320	<4	—	—	6 000	—	—	42
Colif + Staph aur	β h streptoc. + Staph aur	640	>4	16	—	16 800	24	19	115
—	—	160	4	—	—	6 600	17	14	22
	—	10	4	—	—	6,200	—	—	50
—	—	320	>4	—	—	7 200	25	35	45
—	—	160	4	—	—	7 000	17	16	8
—	—	<5	<4	—	—	6,500	—	—	44
—	—	20	<4	—	—	6 800	28	25	48
	—	80	>4	—	—	6 600	—	—	42
	—	40	<4	—	—	5 200	—	—	76
	—	20	<4	—	—	4 000	—	—	32
	—	—	—	—	—	5 400	45	20	36
—	Pneumococc	—	—	—	—	3,500	28	24	4

Group I represents seven patients with marked ECG changes Group II 1—II 6 represent patients with moderate ECG changes and group II 7—II 11 patients with normal ECG

diphtheria in 1918 and had suffered from precordial pain and palpitations in the last few years Case II 5 (45 years) had neurotic heart symptoms in 1961 Case II 10 (72 years) had arterial hypertension

TABLE 22 Anthropometric data heart volume total haemoglobin blood volume and working capac exercise test Time after vaccination given in table Classification of ECG I—Normal ECG

Patient group no	Sex	Age years	Days after vacc	Height cm	Weight kg	Heart volume ml
I 1	♂	66	44	182	84	935
I 2	♂	25	64	187	60	728
I 3	♂	39	46	173	85	835
I 4	♂	20	46	175	71	940
I 5	♂	21	64	177	68	780
I 6	♂	38	36	178	71	835
I 7	♂	20	72	180	78	780
II 1	♀	25	32	164	50	520
II 2	♂	35	111	167	56	744
II 3	♂	44	19	182	72	970
II 4	♀	58	28	161	59	760
II 5	♀	44	60	165	63	640
II 6	♀	12	95	161	46	408

Methods

On admission the history was recorded and a physical examination was performed. Cultures from the nasopharynx were taken in all cases on the first day in hospital, and in patients with suppurating lesions, pus from these being sent for culture (7). HIT was determined at least once in all patients except case 3 in group I and cases 10 and 11 in group II.

SGOT, SGPT and ESR were determined on admission. Phonocardiograms were recorded in all patients with abnormal physical findings in the clinical examination.

ECG was recorded at rest in standing position and during exercise on a bicycle ergometer in sitting position. At rest leads I, II, III, aVR, aVL, aVF, $CR_{1,4,6,7}$ and $V_{1,3,4,5,7}$ were used in standing position only. I, II, III, CR_{1-7}. During exercise ECG was recorded after 5 minutes work at each load. The indifferent electrode was shifted from right arm or central terminal to the forehead, and so called CH leads $CH_{4,5}$ were obtained. CH leads have been shown to be approximately identical to CR leads (16).

Exercise tolerance test. The patients exercised on an electrically braked ergometer in sitting position starting with a load of 200 kpm/min for women and 300 kpm/min for men. The exercise was continued for six minutes at each load and the load was then increased in steps of 200 or 300 kpm/min respectively up to a heart rate of 170 beats/min. The rate of work, the patient could perform at a heart rate of 170 beats/min (W_{170}), was taken as a measure of (stroke volume x the arteriovenous oxygen difference) and was used for cor

ity in 13 patients with pathologic ECG after small pox vaccination on the occasion for the first
II = Slightly different from normal, III = suspected abnormal, IV = abnormal ECG

Total haemoglobin g	Blood volume l	$W_{1.4}$ kpm/min	Highest load kpm/min	beats/min	ECG diagnosis Rest	Exercise
825	5 7	—	900	124	IV	III
745	4 8	850	900	177	III	IV
7a1	5 3	875	900	174	IV	IV
805	5 2	1,250	1,200	166	IV	III
675	4 7	1 000	900	164	IV	III
6i0	4 4	900	1 200	170	I	III
720	5 3	6?0	600	164	IV	I
457	3 3	400	600	172	I	II
635	4 6	6a0	600	16a	I	II
—	—	—	—	—	IV	—
—	—	—	—	—	IV	—
560	4 5	550	600	1?0	III	III
400	3 1	600	600	168	I	II

relation with various anthropometric parameters (25)

Total haemoglobin (THb) in grammes was determined by the alveolar CO method (24) and *blood volume* (lit) was calculated by dividing THb by haemoglobin concentration *Heart volume (HV)*, in ml was determined roentgenologically in prone position according to Larsson and Kjelliberg (19) Normal values of $W_{1.4}$, THb and HV used for comparison were those presented by Holmgren et al (15)

Results

Symptoms All cases except 4 and 7 in group I suffered from oppression Three patients (1 3 and 5) complained of precordial and retrosternal pain One case I 5 had palpitations (Table 21) In group II four patients had dyspnoea, three a feeling of oppression and one patient had palpitations Case II 9, a woman 35 years of age had all symptoms listed in Table 21 but all tests — ECG, heart volume and exercise tolerance — were normal Five patients in group II had no symptoms at all

Symptoms were thus present mainly in the group with marked ECG changes (group I) and consisted of oppression dyspnoea precordial and retrosternal pain

Auscultation On daily auscultation a pericardial friction rub was found in case I 1 Case I 5 had a marked third heart sound In group II a friction rub was observed in case 7 and embryocardia in case 5

White cell count showed a slight leukocytosis in 5 cases in group I (Table 21) but none in group II

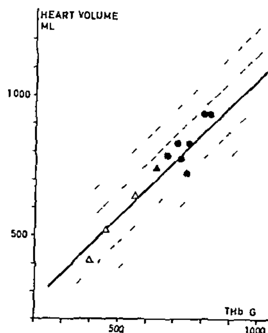


Fig 53 Heart volume (ordinate) in ml in relation to total haemoglobin (abscissa) in g, in eleven patients with abnormal ECG after smallpox vaccination Filled symbols represent men, open symbols, women Circles represent group I and triangles group II The fully drawn line indicates normal relationship between heart volume and total haemoglobin reported by Holmgren et al (15)

Serum transaminases, SGOT and SGPT, were slightly elevated in case I 3 but were not determined in cases I 6, II 2, II 6, II 7, II 8 and II 9

ESR 5/7 cases in group I had elevated values, cases I 3 and I 4 both had normal values In group II there was also a slight to moderate ESR except in cases II 5 and II 11

Bacteriological cultures from the naso pharynx showed in group I a growth of Staphylococcus aureus in 4/7 cases, pneumococcus in one case and β haemolytic streptococci in one case Culture from the local lesion showed growth of Staphylococcus aureus in three patients, β haemolyzing streptococci in one and coliforms in one patient In group II, culture from nasopharynx showed growth of pneumococci in one isolated case, all others had negative cultures both from nasopharynx and local lesion

Haemagglutination tests were positive in a titre of up to 1 40 or more in 5/6

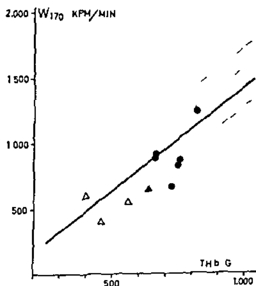


Fig 54 Rate of work that can be performed at a heart rate of 170 beats per minute (W_{170}, kpm/min in relation to THb in g Material and symbols as in Fig 1

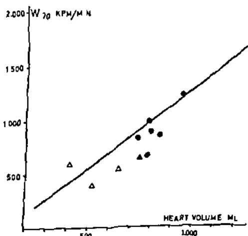


Fig 55 W_{170} kpm/min in relation to heart volume in supine position in ml Material and symbols as in Fig 1

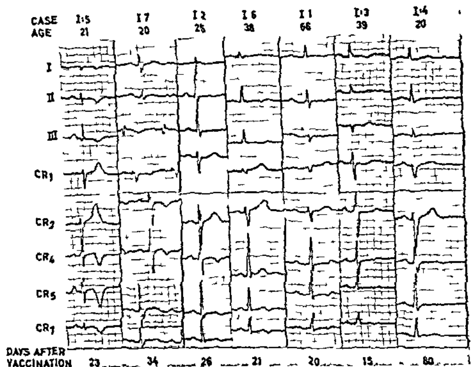


Fig 56 ECG recordings with the most marked deviation from normal in the course of a pathological ECG reaction to smallpox vaccination in seven patients in group I The ECG have been mounted with the cases with most marked changes to the left and with slight changes to the right

cases in group I and in 5/9 cases in group II A fourfold or greater increase of the titre in repeated analyses was observed in 2/4 cases in group I and 3/9 cases in group II

Comment on results of bacterial and immunological data Of the patients in group I 5 7 had a bacterial infection in the local lesion and/or a bacterial colonization of the nasopharynx accompanied by leukocytosis A positive culture was obtained only from the nasopharynx in one patient in group II

Total haemoglobin (g) varied between 0 88 and 1 29 g kg body weight in group I and between 0 82 and 1 13 in group II These values are of the same order as presented by Holmgren et al (15) (Table 23)

Heart volume (ml) in prone position varied between 728 and 940 ml in group I and between 408 and 970 ml in group II In Figure 53 heart volume is related to THb and is found to lie within the normal range of variation in all cases (15) (Table 23)

Exercise tolerance expressed as the rate of work that could be performed at a heart rate of 170 beats/min W_{170}, varied between 680 and 1 250 kpm/min in group I and 400 and 650 in group II W_{170} is related to heart volume and total

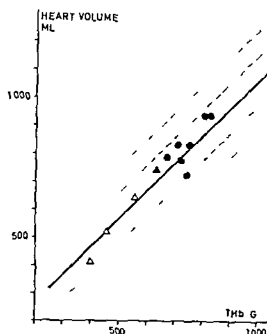


Fig 53 Heart volume (ordinate) in ml in relation to total haemoglobin (abscissa) in g in eleven patients with abnormal ECG after smallpox vaccination Filled symbols represent men open symbols women Circles represent group I and triangles group II The fully drawn line indicates normal relationship between heart volume and *total haemoglobin* reported by Holmgren et al (15)

Serum transaminases, SGOT and SGPT, were slightly elevated in case I 3 but were not determined in cases I 6, II 2, II 6, II 7, II 8 and II 9

ESR 5/7 cases in group I had elevated values, cases I 3 and I 4 both had normal values In group II there was also a slight to moderate ESR except in cases II 5 and II 11

Bacteriological cultures from the nasopharynx showed in group I a growth of Staphylococcus aureus in 4/7 cases, pneumococcus in one case and β haemolytic streptococci in one case Culture from the local lesion showed growth of Staphylococcus aureus in three patients, β haemolyzing streptococci in one and coliforms in one patient In group II, culture from nasopharynx showed growth of pneumococci in one isolated case, all others had negative cultures both from nasopharynx and local lesion

Haemagglutination tests were positive in a titre of up to 1 40 or more in 5/6

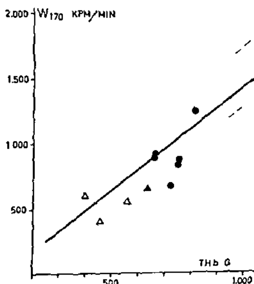


Fig 54 Rate of work that can be performed at a heart rate of 170 beats per minute (W_{170} kpm/min in relation to THb in g Material and symbols as in Fig 1

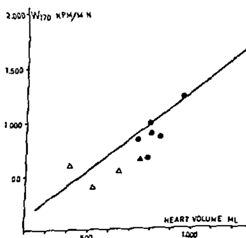


Fig 55 W_{170} kpm/min in relation to heart volume in supine position in ml Material and symbols as in Fig 1

may have occured one week earlier In group II an abnormal ECG was recorded after 10—20 days in four patients, and in one (case II 5) after 31 days In cases I 1 and I 4 the first ECG recorded was normal and here the onset can be fixed to have occurred between 10 and 20 days after inoculation In cases I 2 I 3 I 5, I 6, II 2 and II 6 the first ECG recorded on the tenth day after vaccination was abnormal In three of them I 2 I 5 and I 6 the abnormality consisted of abnormal S T elevation indicative of pericarditis in leads II—III CR_1, CR_3 and CR_5

The abnormal ECG changes were dominated by the occurrence of a pathological T vector resulting in negative or flattened T waves most commonly in leads I II and CR_4—CR_7 in 6/7 patients in group I and in CR_1, CR_2 and CR_4 in case I 7 (Fig 56 and 57)

As stated above the material was grouped according to the severity of ECG changes while the subjects were in hospital The severity was judged to be the magnitude of the most negative T wave there was some slight overlapping between the groups Maximum abnormality occurred between 20 and 80 days after vaccination in group I and between 10 and 21 days in group II The ECG remained abnormal for 46 days — 5 months in group I and 1—85 days in group II (Figs 56 and 57)

The ECG during exercise was recorded between 44 and 72 days after the vaccination in group I and after 19—111 days in group II (Table 23) The resting ECG before the exercise test was classified as abnormal in cases I 1, I 3, I 4 I 5 and as suspected abnormal in I 2 and II 5 During exercise the ECG remained abnormal in I 3 and was classified as abnormal in case I 2 and suspected abnormal in I 1, I 4, I 5 I 6 and II 5 The ECG changes on which this classification was based were aberrant conduction of single beats in one case (I 2) and ST-T changes with or without a positive after potential in leads CH_4 and CH_7 in all cases (15) No case had a negative T wave in the recorded leads (CH_2—CH_7) during exercise

Discussion

The classification of the material into two groups according to the degree of ECG changes was done before the results of the bacterial cultures were available Group I was then found to consist only of men with, in the majority of cases positive bacteriological culture from local lesion and nasopharynx In group II the majority of the cases were women (8/11) Similar observations on the sex distribution of patients into ECG changes suggestive of myocardial lesion have been reported earlier in children (5 20) but not in adults The clinical symptoms of manifest infection were leukocytosis in 5/7 cases furunculosis in one case erysipelas in one case and septicaemia in one case

In the rest of the subjects the nasopharyngeal infection was subclinical

The coincidence between positive bacteriological cultures from lesion and nasopharynx and the severity of ECG change is striking and a causal rela

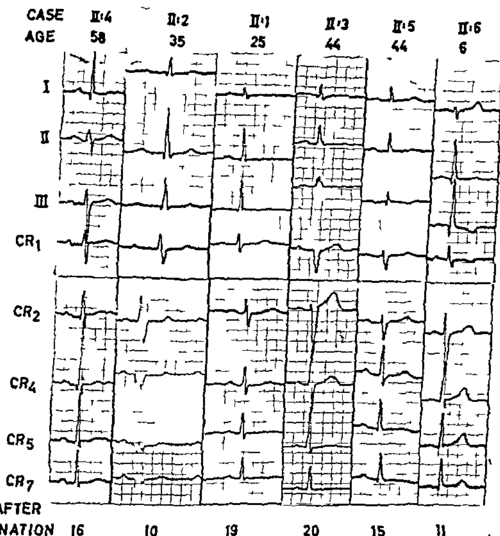


Fig 57 ECG recordings with most marked deviation from normal in the course of a pathological ECG reaction to smallpox vaccination in the six patients with ECG changes in group II Order of mounting as in Fig 4

haemoglobin (15) in Figs 54 and 55 and is seen to fall below — SD in cases 3 and 7 in group I and in cases 1, 2 and 5 *in group II* In 4/6 patients in group I and 3/4 in group II, $W_{1,0}$ is lower than the normal value predicted from THb and heart volume In 5/7 patients the ECG at rest on the same occasion was abnormal or suspected abnormal and in 6/7 in group I the ECG during exercise was abnormal or suspected abnormal In group II the corresponding figures were 3 6 and 1/4 (*cf* Table 22)

ECG at rest The abnormal ECG changes, ST elevation or abnormal *T* vector were first recorded between 10 and 34 days after vaccination mean interval 16 6 days (*cf* Table 23 In group I an abnormal ECG was recorded within 10 days after vaccination in 4/7 patients, in two between 10—20 days and in one case after 34 days In this case *I 7*, the date of inoculation is uncertain and

and the ECG with max abnormality as judged by the negative amplitude of T duration of ECG
slightly *different from normal*) III = suspected abnormal IV = abnormal ECG) in 13 patients with
(ST—T—) indicate here a slight ST-depression accompanied by a flattened T and a so called posi
ing to that published by *Holmgren at al* 1959 T alone signifies a flattening of T ST T indicates

at rest			ECG abnormality on exercise	Classification
Negative T	Flat T	Classification		
I II CR_{4-7}		IV	ST—T CH_{5-7}	III
II CR_{4-7}	I	IV	Vent. aberration T CH_{5-7}	IV
CR_7	I CR_{2-5}	IV	ST—T CH_{5-7}	IV
	CR_{4-7}	IV	ST T CH_{5-7}	III
I III CR_{4-7}		IV	CH_{5-7}	III
CR_{5-7}	II III	IV	ST T CH_{5-7}	III
CR_{1-4}	II CR_7	IV		I
	I–III CR_{4-7}	IV	ST–T CH_{5-7}	II
CR_7	I CR_{4-5}	IV	ST T CH_{5-7}	II
	I–II CR_{5-7}	IV		
CR_{-4}	I II CR_1 CR_{2-7}	IV		
CR_1	II	III	ST T CH_{5-7}	III
III CR_1	II	III	ST-T CH_{5-7}	II

beats per minute (Figs 54 and 55) was somewhat low in 6/11 cases in relation *to THb and in* 5/10 *cases in relation* to heart volume The size of the heart in supine position was not increased in any patient in whom it was measured

A decrease of W_{170} with the circulatory dimensions THb and heart volume as found in the present material can be caused by a hyperkinetic circulation (15) or a decrease of the stroke volume that can be maintained during exercise Such a decrease of stroke volume can be caused by impaired filling of the heart in diastole due to orthostatic blood shifts in sitting *position (9) or to impaired con*tractility of the myocardium as a result for instance of a myocardial lesion. The investigations performed do not allow any conclusion as to the cause in the present material However all patients were immobilized and partly confined to bed for at least 19—111 days i e the duration of the ECG changes, and it seems reasonable to assume that part of the decrease in W_{170} was caused by inactivity An interpretation of the ECG changes as due to myocarditis is sustained by histological *findings in pa*tients with fatal outcome (10) The clinical diagnosis of myocarditis is always more or less based on ECG changes but it is important to remember that little is yet known of the relationship between amplitude of ECG change and severity of lesion The grouping of the

TABLE 23 Time interval, days between small pox vaccination and the first recorded abnormal ECG abnormality localisation and classification of max ECG changes (I=normal ECG II=abnormal ECG after small pox vaccination The exercise ECG changes characterized as tive afterpotential (17) The classification of these changes is made by a scale correspond ST depression and a flattening of T

Case	Interval days, vaccination to		Duration of ECG abnormality	ECG abnormality
	1st abnorm ECG	Max ECG abnorm		ST elevation
I 1	15	20	5 months	
I 2	10	26	70 days	II, III CR_{1-},
I 3	10	15	53 days	
I 4	18	80	46 days	
I 5	10	23	119 days	CR_{1-7}
I 6	11	21	101 days	II III CR_{4-7}
I 7	34	34	53 days	
II 1	19	19	35 days	
II 2	10	10	24 days	
II 3	19	20	34 days	
II 4	18	16	36 days	
II 5	31	15−21	85 days	
II 6	11	11	11 days	

tionship cannot be excluded Large-scale investigations of the frequency of ECG changes in different clinical β-haemolytic streptococci infections have shown that ECG changes occur only in patients with clinical symptoms and signs of disease, not in carriers (4) It therefore seems reasonable to conclude that the myocardial lesions in the present series are not due to the bacterial findings in skin and nasopharynx alone though this may have been of etiological importance

The ECG changes were recorded approximately simultaneously with the appearance of symptoms such as oppression, precordial pain and dyspnoea, and seem to be well correlated to these clinical manifestations, as has also been found earlier (5)

Symptoms were absent in two patients in group I, one of whom (case I 7) was stuporous when the ECG changes occurred The incidence of symptoms in group II was much lower (6/11) Only one of these patients had a pathological ECG The symptoms lasted only a short time in all cases, while abnormal ECG continued for a considerable period in group I

The exercise tolerance tests were performed before normalization of the ECG at rest since it was desired to evaluate the circulatory significance of the ECG changes The working capacity expressed as the rate of work at a heart rate of 170

and the ECG with max abnormality as judged by the negative amplitude of T, duration of ECG slightly different from normal III – suspected abnormal IV = abnormal ECG) in 13 patients with (ST–T+) indicate here a slight ST-depression accompanied by a flattened T and a so called posi ing to that published by Holmgren at al 1959 T alone signifies a flattening of T ST T indicates

at rest				
Negative T	Flat T	Classification	ECG abnormality on exercise	Classification
I II CR_{4-7}		IV	ST T CH_{5-7}	III
II CH_{4-7}	I	IV	Vent aberration T CH_{5-7}	IV
CR_7	I CR_{2-5} CR_{4-7}	IV IV	ST-T CH_{5-7} ST-T CH_{5-7}	IV III
I III CR_{4-7}		IV	CH_{3-7}	III
CR_{3-7}	II III	IV	ST-T CH_{5-7}	III
CR_{1-4}	II CR_7	IV		I
	I III CR_{4-}	IV	ST-T CH_{5-7}	II
CR_7	I CR_{4-5}	IV	ST T CH_{5-7}	II
	I II CR_{5-7}	IV		
CR_{-4}	I II CR_1 CR_{5-7}	IV		
CR_1	II	III	ST T CH_{5-7}	III
III CR_1	II	III	ST T CH_{-7}	II

beats per minute (Figs 54 and 5a) was somewhat low in 6/11 cases in relation to THb and in 5/10 cases in relation to heart volume The size of the heart in supine position was not increased in any patient in whom it was measured

A decrease of W_{170} with the circulatory dimensions THb and heart volume as found in the present material can be caused by a hyperkinetic circulation (15) or a decrease of the stroke volume that can be maintained during exercise Such a decrease of stroke volume can be caused by impaired filling of the heart in diastole due to orthostatic blood shifts in sitting position (9) or to impaired contractility of the myocardium as a result for instance of a myocardial lesion The investigations performed do not allow any conclusion as to the cause in the present material However all patients were immobilized and partly confined to bed for at least 19—111 days i e the duration of the ECG changes and it seems reasonable to assume that part of the decrease in W_{170} was caused by inactivity An interpretation of the ECG changes as due to myocarditis is sustained by histological findings in patients with fatal outcome (10) The clinical diagnosis of myocarditis is always more or less based on ECG changes, but it is important to remember that little is yet known of the relationship between amplitude of ECG change and severity of lesion The grouping of the

material according to deviation from normal should not necessarily be taken to mean that the most marked ECG changes are correlated to the largest lesion. Indicative of the clinical diagnosis of myocarditis are the variation in the ECG picture followed by a complete restitution to the pattern before the lesion, provocation of abnormal ECG in convalescence by exercise tests, changes in heart size and working capacity, and physical signs such as friction rub. Based on such considerations it is our opinion that group I probably consists mainly of a material with postvaccinal myocarditis. The possible presence of extracardial factors, such as vasoregulatory asthenia, orthostatism, and earlier heart disease, makes the final diagnosis difficult in group II. In two patients pericardial friction rub was auscultated, a finding which supports the diagnosis of myo pericarditis.

The clinical course of the complication seems to be established in this material. The myocarditis appears within 1—3 weeks after the inoculation. The symptoms and signs last as a rule only about a week, but the ECG changes between 2 and 5 months.

The aetiology of postvaccinal myo carditis has earlier been discussed as being based on a viraemia (7, 21) or a general hypersensitivity reaction (11, 21). The former hypothesis is supported by the view that eczema vaccinatum is caused by an insufficient production of antibodies during viraemia and by the observation that myocardial complications have appeared one day after the inoculation. In this material the interval between inoculation and ECG changes is much longer, this does not contradict the former theory, however, as viraemia has been reported 100 days after inoculation (1). Eczema vaccinatum was present in two cases in group I and one case in group II.

The interval between inoculation and the onset of ECG changes has earlier been reported to vary between 5 days to 3 weeks. In this material most changes started within 1—3 weeks, an interval compatible with the hypothesis of a hypersensitivity reaction. Coronary thrombosis has been reported after smallpox vaccination in 5 elderly patients (53—88 years) 7—14 days after the inoculation (22). An ECG picture suggestive of coronary thrombosis has been reported 7—14 days after treatment with antitetanus serum (8). It seems obvious that hypersensitivity reactions may participate in the development of the observed myocardial reactions (6). 6/18 patients had other allergic reactions simultaneously.

Summary

192 patients admitted to the Stockholm Hospital for Infectious Diseases for various postvaccinal complications were studied with repeated ECG recordings.

The patients with abnormal ECG (11/18) or marked symptomatology suggestive of heart complications (7/18) were studied with repeated ECG exercise tests and determination of heart volume and total haemoglobin.

The results were related to bacteriological, serological and clinical findings. The abnormal ECG appeared within 3 weeks. Seven patients with marked

ECG changes formed group I and II patients with moderate ECG changes or marked symptoms group II Group I consisted of men only 5/7 had positive bacteriological cultures from the lesion or nasopharynx

Group II consisted of 3 men and 9 women Only one patient in this group had bacteria in nasopharynx The ECG changes lasted between 11 days to 5 months

Heart volume and total haemoglobin were normal in all subjects There was a tendency to low working capacity in relation to THb and heart volume

It is inferred that secondary bacterial infections may play role in the aetiology of acute myo pericarditis The factors of viraemia and delayed hypersensitivity is discussed

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From the Department of Clinical Physiology (Head Prof T Sjöstrand M D) and the Military Medical Examination Centre (Swedish Defence Medical Board Head Surgeon General C J Clemedson M D) Karolinska sjukhuset Stockholm Sweden

ECG-Changes without Subjective Symptoms after Smallpox Vaccination of Military Personnel

Björn Ahlborg, Klas Linroth and Bengt Nordgren

Introduction

Vaccination against small pox is carried out in the childhood on almost the whole population in Sweden This vaccination has, however, been followed by secondary complications from different organs in the body some of which must be regarded as very serious i e post vaccinia encephalitis (4) When beginning military service all conscripts are vaccinated again t small pox again.

When starting military service many of the young men have a fairly bad physical working capacity (12 5 15) They are therefore trained in a rather intensive way in the beginning which implies a demand on the cardiovascular system among others The vaccination procedure is thus carried out at the same time as the demand on the cardiovascular system is comparatively high In fact reports have appeared of changes in the ECG in connection with vaccination against small pox (9 12)

The purpose of the present investigation was to study if there was any connection between changes in the function of the heart and vaccination against small pox in young men during military service As a matter of fact this offered an opportunity to obtain a group of men which could be followed up comparatively easily

Material

320 conscripts were studied Among these 286 were studied both immediately before and about 3 weeks after vaccination against small pox (vaccine No E 56, extract from chicken made by National Bacteriological Laboratory Stockholm) The rest was studied only before or after vaccination and was therefore excluded from the main material Before the vaccination (simultaneously with vaccination against small pox vaccination was made against tetanus and — with those who were tuberculine negative — against tuberculosis) they had been in military service for only a few days (regiment KA 1 Vaxholm, Sweden) Most of the conscripts were 19 years old They were recruited to a big extent from Stockholm and are therefore hardly representative for the whole population in Sweden Those, in whom the first vaccination failed, were revaccinated later and in this group ECG registration was made about ten days after the second vaccination The study

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TABLE 25

Subj	ECG changes before vaccination — Recumbent	ECG changes before vaccination — After stand 8 min	ECG changes after vaccination — Recumbent	ECG changes after vaccination — After stand 8 min
B 299	HR: 80–60 Incomp RBBB No ST T changes	HR 90 ECG unchanged	HR 80 T neg T II III flat CH_4 CH_7	HR 85 T d phas T II and CH_4 neg III CH_4 CH_7
M 019	HR 65 No ST T changes	HR 90 No ST T changes	HR 70 T neg CH_4 CH II and III	HR 100 The same ECG changes
T 123	HR 85 PR 0 18 No ECG changes	HR 105 No ECG changes	HR 85 PR 0 17 A few premature atrial beats Partial atr ventr block (3 beats/17 blocked)	HR 75 PR 0 16 No atrioventr block registered
N 895	HR 75 S nus rythm and ectop c atrial rythm Incompl BBB T flat corresp to LV	HR 100 Only s nusrythm. T more pronounced neg corr to LV	HR 90 Ectop c atrial rythm A little more pron neg T corr to LV	HR 105 S nusrythm The same ECG changes as before vacc (In stand position)
N 087	Slight s nusarrythmia (80 100) T d phas c neg corr to LV	ECG unchanged	ECG unchanged	ECG unchanged
O 005	HR 60 PR 0 37	HR 60 PR 0 33	HR 85 PR 0 33	HR 105 PR 0 33
I 163	HR 122	HR 125	HR 125	HR 135
S 005	Incomplete BBB	No further remarkable ECG changes	ECG unchanged	No further remarkable ECG changes

HR Heart rate CH Cheast Head lead LV = Left Ventricle BBB = Bundle branch block

) determination of total haemoglobin (14) classification by Holmgren et al (7) Results from the study are found in Tables 24—26

Results

The data obtained were programmed for handling in a data machine The interpretation of the ECG was transformed through a modification of the Minnesota code (1) The ECG changes after standing were treated according to the

Discussion

ECG before and after vaccination against small pox was recorded in 286 conscripts during their first military service

On account of the ECG changes recorded eight men were considered to

Table 24

	No	Yes
Anamnesis before vaccination		
Heart disease	279	0
Limitated in gymnastics or physical exercise	273	6
Fainted earlier	273	6
Tachycardia any time	275	4
Pains in the chest	274	5
Shortness of breath after light physical exercise	277	2
Collaps during physical exercise	278	1
Illness with fever during the last month	232	47
Scarlet fever	226	53
Any rheumatic disease	276	3
Vaccinated against small pox earlier	10	268
Are there any scars of this vaccination left	12	266
Anamnesis after vaccination		
Local reaction with carify at the first vaccination	14	272
Swelling of the lymph glands in the axill	78	208
Fever after the vaccination	225	61
Have you caught a cold after the vaccination	70	214
Any discomfort from the heart after the vaccination	278	8
Is your physical condition worse after the vaccination	249	36

took place during January and February in 1963 In fact everybody was vaccinated and had got vesicles on the place for vaccination when the second part of the ECG-registration procedure was made

Methods

The study can be divided into 3 parts

1 *Anamnesis* Every man had to answer certain questions, which were considered to be of possible interest for the study

2 *ECG* was taken with a direct writing four channel apparatus (type Elema B 42) The leads used were standard leads (I, II and III) and precordial leads CH_{I}, CH_{II}, CH_{IV}, CH_{V} and CH_{VI} Before and after vaccination ECG was taken both with the subject recumbent and after standing eight minutes

3 When the ECG s obtained were interpreted, some of the subjects were called back for further examination This consisted thus in selected cases of

1) Clinical examination

2) ECG (the same leads as before, and in some cases even Wilson leads) in recumbent position and after standing during 8 minutes

3) Test of the physical working capacity (ad modum K S, *13, 16*) on an ergometer bicycle *(8)*

4) determination of heart volume with X ray technique (11)

TABLE 25

Subj	ECG changes before vaccination: Recumbent	ECG changes before vaccination: After stand 8 min	ECG changes after vaccination: Recumbent	ECG changes after vaccination: After stand 8 min
B 299	HR 80–90 Incomp RBBB No ST–T changes	HR 90 ECG unchanged	HR 80 T neg T II–III flat CH_4–CH_7	HR 85 T diphas T II and CH_4 neg III CH_5 CH_7
M 019	HR 65 No ST–T changes	HR 90 No ST–T changes	HR 70 T neg CH_5 CH_7 II and III	HR 100 The same ECG changes
T 123	HR 85 PR 0 18 No ECG changes	HR 105 No ECG changes	HR 85 PR 0 17 A few premature atrial beats Partial atr ventr block (3 beats/17 blocked)	HR 75 PR 0 16 No atrioventr block registered
N 895	HR 75 Sinus rythm and ectopic atrial rythm. Incompl BBB T flat corresp to LV	HR 100 Only sinusrythm T more pronounced neg corr to LV	HR 90 Ectopic atrial rythm A little more pron neg T corr to LV	HR 105 Sinusrythm The same ECG changes as before vacc (In stand. position)
N 087	Slight sinusarrythmia (80–100) T diphasic neg corr to LV	ECG unchanged	ECG unchanged	ECG unchanged
O 095 L 163 S 005	HR 60 PR 0 37 HR 122 Incomplete BBB	HR 60 PR 0 33 HR 125 No further remarkable ECG changes	HR 85 PR 0 33 HR 125 ECG unchanged	HR 105 PR 0 33 HR 135 No further remarkable ECG changes

HR Heart rate CH = Cheast Head lead LV = Left Ventricle BBB = Bundle branch block

5) determination of total haemoglobin (14)

Results

The data obtained were programmed for handling in a data machine The interpretation of the ECG was transformed through a modification of the Minnesota code (1) The ECG-changes after standing were treated according to the classification by Holmgren et al (7) Results from the study are found in Tables 24 –26

Discussion

ECG before and after vaccination against small pox was recorded in 286 conscripts during their first military service

On account of the ECG changes recorded eight men were considered to

Table 26

Subj	THb g		Hb conc g/100 ml		Blood vol l	
	Est	Found	Est	Found	Est	Found
B 299	850	905	15 4	13 4	5 5	6 8
M 019	730	735	15 4	13 7	4 7	5 4
F 123	965	795	15 4	14 8	6 3	5 4
N 895	720	610	15 4	14 0	4 7	4 4
N 087	985	755	15 4	15 0	6 4	5 0
Ö 005	750	605	15 4	13 2	4 9	4 6
E 163						
S 005	670	625	15 4	14 4	4 4	4 3

[1] HR 95 PQ 0 38 T flat-diphasic I-III CH, CH Signs of myocardial lesion corresponding to L V After standing 8 min HR 95 PQ 0 19 During work normal PQ time (PQ 0 15 HR 170) and pos T wave After the working test partial A V block (A V block II) with Wenckebach periods

need further examination This was done, because it is necessary to relate the changes in the ECG s registered to the function of the circulatory system and especially that of the heart ECG-changes without any following disturbance of the heart function is of course of no practical importance for the patient Thus the ECG must be considered more as a guide than as real measure of the dynamics of the heart function As, however, the registration of the ECG is a comparatively quick procedure compared with most of the methods used in Table 26, this is a suitable way for screening the material Heart function changes without concomitant changes in the ECG registrations may thus not be detected if the patients do not consult the physician later It also follows that ECG changes without probable connections with heart function changes after vaccination were not followed up

An example of this way of discussion was that no further studies were made on one subject with an ECG record typical for preexcitation but without any subjective symptoms (among others no attacks of tachycardia)

The scope of the study was to investigate the frequency of possible heart complications due to vaccination against small pox, especially as no more detailed surveys — in the knowledge of the authors — on this topic have been made The subject has, however, been studied

Lagerlöf (9) and *Linroth* (*12*) suspected a connection between vaccina-

Hert vol recumbent ml		Physical work capacity at HR 170/min kpm/min (=PWC₁)		ECG reaction recumbent and after standing 8 min	ECG reaction during the excercise test
Est	Found	Est	Found		
975	870	1380	1100	No change	No change
805	750	10/0	1000	No change	No change
1060	1000	1500	1150	*	*
67ɔ	/80	840	950	*	*
825	—	1100	—	—	—
6ɔ0	790	850	900 (HR 152 not steady state)	HR 6ɔ PQ 0 38	HR 150 PQ 0 33
	810		900	HR 104 After standing 8 min HR 125	No change
690	680	860	600 (not steady state)	HR 72 After standing 8 min HR 124	No change

* At rest ectopic atrial rhythm After standing 8 min disappearance of the ectopic rhythm sympaticoton ECG reaction During exercise ECG reaction without remarks.

tion against small pox and signs of myocarditis on ECG after the vaccination Bengtsson and Lundstrom (2) and Holmgren et al (6) have been recording ECG in patients with clinical complications following small pox vaccination Gunnert and Morsing have shown ECG alterations in conscripts after vaccination (3)

In our material 61 subjects reported fever after vaccination i e more than 1 5 had signs of a more generalized reaction As a comparison the frequency of encephalitis post vaccination has been reported to about 1 100 000 to 1 15 000

There is also the question of the time course of myocardial lesions (i e the time after the vaccination when possible changes could appear be most pronounced and disappear) and our possibilities to diagnose them with the methods used

As most cases of myocarditis have a very benign clinical course it should be quite possible that some more or less insignificant myocardial lesions could occur after small pox vaccination In young healthy men most of these possible changes might be expected to pass without clinical signs and therefore possibly only registered if ECG is taken

In this study 3 subjects (B 299 M 019 T 123) showed changes in the ECG after vaccination which could be interpreted as myocardial lesions due to the vaccination

One subject (N 89ɔ) had already before the vaccination a pathological ECG The ECG changes in subject N 89ɔ

(mainly in the T-wave region) were a little more pronounced after the vaccination The degree of difference before and after might, however, be due to the variations seen in some cases from one time to another without any concomitant demonstrable changes in the function of the heart

The changes in subject N 895 were therefore interpreted as most likely to be due to 'the ordinary variations of the ECG-picture"

Four cases (N 087, Ö 005, E 163 and S 005) had already before the vaccination changes in the ECG These were not affected by the vaccination

Of these eight cases six were found to have definitely pathological ECG registered while the two remaining subjects (E 163 and S 005) were classified as not certainly pathological changes

None of these subjects had any subjective symptoms of heart disease of such a degree that they consulted the physician in charge on the regiment The possibility of 'silent" heart disease during a period of hard physical training makes it therefore important to evaluate the heart function in these cases The *cases will now be discussed in more detail* (Tables 25 and 26)

B 299 — Vaccinated for the first time in his life After vaccination appearance of signs of myocardial lesion (isolated neg T-wave in leads corresponding to the left ventricle) No subjective symptoms of disease No fever reaction after *the vaccination* Control 3 months after vaccination showed complete regress of the T-wave changes A thorough test of the cardiovascular function both about 7 months and one year after the vaccination showed no certain abnormal *signs of the cardiovascular function*

Conclusion Myocarditis after vaccination which healed without any demonstrable changes

M 019 — Before vaccination normal ECG After vaccination flat neg T-wave corresponding to the left ventricle Not vaccinated against small pox before the military service 8—9 days after the vaccination fever reaction Also anamnesis of upper respiratory infection No bed rest Control after 1 1/2 month showed *unchanged ECG Controls after* about 7 months and one year showed complete regression of the ECG changes and a normal heart function

Conclusion Myocarditis after vaccination, which healed without any demonstrable changes The respiratory infection cannot be excluded to have con *tributed to the disease*

T 123 — After vaccination appearance of rhythm disturbances recorded in ECG No fever No respiratory infection Control after about 3 months status quo" Control after 7 months still no subjective symptoms but a) more pronounced rhythm disturbances and b) *appearance of neg* T wave corresponding to the left ventricle, c) pathological ECG reaction at the working test (rhythm disturbances) Control one year after vaccination still showed no subjective symptoms and no regression of mentioned findings

Conclusion Signs of 'silent myocarditis after vaccination which were even more pronounced both 7 months and one year later and also affected the dynamics of the heart function From a clinical point of view a very illustrative

case that probably would not have been detected unless ECG had been taken

N 895 — FCG changes both before and after the vaccination Fever reaction after the vaccination and also symptoms of mild respiratory infection No anamnesis of heart disease or previous infections Control after 3 months showed partial regression and after 7 months complete regression of the T wave changes and a normal working test

Conclusion 'Silent' ECG changes which can be seen in connection with the vaccination

V 087 — ECG-changes present before and not affected by the vaccination No anamnesis of disturbance in the cardiovascular function

Conclusion ECG changes which could be interpreted as silent myocarditis not caused by vaccination.

Ö 005 — Prolonged time for the atrio ventricular conduction (AV block I) unaffected by the vaccination Since many years periods of subjective fatigue and periods of heart discomfort of such a degree that he had been periodically free from gymnastics during school time Scarlet fever at seven years of age No subjective symptoms from the heart

Conclusion A—V block without connection with the vaccination

E 163 — No signs of myocardial lesion Already at rest a considerable tachycardia for a young man complains of subjective symptoms of uncharacteristic "heart discomfort Further test of the cardiovascular function gave no indications of changes in the cardiovascular function Anamnesis of a considerable alcoholic consumption

Conclusion Tachycardia without connection with the vaccination

S 005 — Incomplete intraventricular block unchanged after vaccination The physical working capacity lower than predicted No subjective symptoms of disease

Conclusion ECG-changes without connection with the vaccination

Summary

In 286 men during military service ECG was recorded in recumbent position and after standing for 6 minutes before and after vaccination against small pox The scope was to study if the vaccination could give myocardial involvement

The cases with any remarkable ECG changes were followed up with

a) clinical examination
b) ECG recording
c) physical working capacity test
d) heart volume determination
e) total body haemoglobin determination

Of the material 3 cases could be interpreted as myocarditis due to the vaccination against small pox Of these 2 had not been vaccinated earlier

3 cases with pathological ECG changes were found without any connection with the vaccination Another 2 cases of remarkable (but not definitively abnormal) ECG registrations were recorded none of which connected with the vaccination

The results of the investigations in this material show to which degree myocardial affections can occur in connection with vaccination against small pox

The frequency of myocardial affections motivate ECG examination on wide indications because pathological ECG changes do occur in not a negligible frequency in 'healthy" conscripts It can be discussed whether ECG should be recorded as a routine procedure before periods of hard physical training even by young people

Acknowledgements

These investigations were carried out at the KA I regiment Vaxholm which has provided facilities for performing the investigations (Dr O Ekenberg Jr) The management of the material in a data machine was made by the Research Institute of National Defence (FOA) which thus supported the study We are also indebted to Surgeon General of the Swedish Army (Dr G Hesselblad) who has also facilitated the investigations

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From the Department of Clinical Bacteriology, Karolinska Sjukhuset Stockholm Sweden

Smallpox Vaccination of Patients with Increased Risk of Complications

Hans Ericsson

On May 18 1963 the National Board of Health issued a statement in which persons for whom smallpox vaccination was desirable but who for various reasons were considered to run an increased risk of complications were advised to attend Karolinska Hospital for assessment prior to possible vaccination *Ad hoc* collaboration was then established primarily between the hospital s central bacteriologic laboratory and its department of dermatology and obstetrics General principles for vaccination were drawn up by representatives of all three departments, with special consideration for rational use of the limited quantities of immune serum globulin that were available Contraindications from general internal medical and radiologic aspects were assessed by the clinical microbiologists all with medical training if necessary in consultation with other specialists The total of cases comprising this varied group was 296 Dermatologic and obstetric contraindications were judged by specialists from the respective departments (G Eriksson M Forsbeck L Eng trom G Ahlin and others)

The risks were discussed at intercollegial conferences and with the individual patients *In addition to conferring* protection against smallpox to directly or indirectly exposed persons the aim was to improve the general immunity among the high risk groups

Previously planned foreign travel was considered to constitute a valid indication for vaccination but occasionally a certificate of exemption from vaccination because of contraindications was issued in appropriate cases In other cases foreign travel, even when previously planned, was strongly discountenanced

Among the 296 cases many widely differing factors were considered as possible contraindications to vaccination (Table 27) In cases with a history of central nervous disorder, demyelinizing processes following morbilli, rubella, pertussis and previous smallpox vaccination were judged to be absolute contraindications whereas encephalitis following poliomyelitis and related diseases was not accepted as a contraindication When the recent medical history included radiotherapy the leukocyte pattern of the blood was one of the *factors* deciding for or against vaccination Corticosteroid treatment contraindicated vaccination if it had been given for an appreciable time and was of the same order of magnitude as the endogenous steroid production Active malignant

The frequency of myocardial affections motivate ECG-examination on wide indications because pathological ECG-changes do occur in not a negligible frequency in "healthy" conscripts It can be discussed whether ECG should be recorded as a routine procedure before periods of hard physical training even by young people

Acknowledgements

These investigations were carried out at the KA 1 regiment, Vaxholm, which has provided facilities for performing the investigations (Dr O Ekenberg Jr) The management of the material in a data machine was made by the Research Institute of National Defence (FOA) which thus supported the study We are also indebted to Surgeon General of the Swedish Army (Dr G Hesselblad) who has also facilitated the investigations

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TABLE 29 Revaccination

Reason for risk	Normal reaction	Local severe reaction	General reaction	*Total*	Immune serum globulin
Cortison treatment	6	2	(2)	*8*	
Radiotherapy	18	1		*19*	(1)
Internal medical disease	49	5	(3)	*54*	(5)
Encephalitis	19		2	*21*	(2)
>60 years old	27	*2*	(1)	*29*	(2)
Total	*119*	*10*	*2* (6)	*131*	(*10*)
Immune serum globulin	(2)	(7)	(1)		(10)

Number in brackets indicates that the patient is also included in an earlier group

stitution therapy for endocrine disturbance Endocrinologic opinion favoured vaccination in this case and no complications occurred Two persons older than 60 years received primary vaccination

Of the applicants for revaccination (Table 29) about two-thirds of those who were being treated with corticosteroids or were above the age of 60 were rejected as were about one third of the patients receiving radiotherapy or who had disorders such as diabetes mellitus asthma circulatory disturbance or malignancy or a history of encephalitis

General reactions to vaccination (pyrexia hedache and general malaise) occurred in two cortisone treated patients who had not been vaccinated at this laboratory but who came to us because of these symptoms A patient with leukaemia was vaccinated before we had formulated our principles for vaccination A severe local reaction appeared and spread over the entire ipsilateral arm She was admitted to the hospital at which her leukaemia was treated Apart from ugly scarring there were no lasting ill effects of vaccination Another patient had been operated on for mammary cancer and after vaccination on the ipsilateral arm numerous secondary vesicles appeared in the axilla There were no other side effects in this case Two patients with a history of inflammatory encephalitis had severe post-vaccinal headache Two elderly patients had severe local reactions to vaccination in one of them with protracted general effects

Immune serum globulin was used only to a small extent and consequently its value could not be objectively assessed In my opinion, immune serum globulin should be reserved mainly for complications of vaccination or for severe reactions Availability of immune serum globulin does not essentially alter the balance between indications for and against smallpox vaccination

In summary the indications for smallpox vaccination were judged against the contraindications in 296 cases in which the medical history indicated

TABLE 27 Applicants for smallpox vaccination

Reason for risk	Primary vaccination		Revaccination		
	Not vaccinated	Vaccinated	Not vaccinated	Vaccinated	*Total*
Cortisone treatment	1	1	20	8	*30*
Radiotherapy	2		12	19	*33*
Internal medical disease	4	1	38	54	*97*
Encephalitis	2	3	13	21	*39*
>60 years old	13	3	52	29	*97*
Total	*22*	*8*	*135*	*131*	*295*

TABLE 28 Primary vaccination

Reason for risk	Normal reaction	Local severe reaction	General reaction	*Total*	Immune serum globulin
Cortison treatment	1			*1*	(1)
Radiotherapy					
Internal medical disease	1			*1*	
Encephalitis	2	1		*3*	(1)
>60 years old	1	2	(1)	*3*	(1)
Total	*5*	*3*	*(1)*	*8*	(3)
Immune serum globulin	(2)	(1)			(3)

Number in brackets indicates that the patient is also included in an earlier group

processes, particularly systemic diseases, were as a rule regarded as precluding vaccination, chiefly because it was felt that deterioration could rightly or wrongly be attributed to vaccination In cases of circulatory disorder or diabetes mellitus attempts were made to estimate whether or not the patient could *without* undue risk tolerate a moderately severe infection, thereafter the indications were weighed against the contraindications For persons more than 60 years old the previous vaccination history formed the basis for judging the advisability or *otherwise* of revaccination

Vaccin of ordinary strength and the multiple pressure method were used for inoculation When necessary the vaccination was repeated until a positive result was obtained

Each applicant for vaccination was given a questionnaire to fill in The answers guided the assessment of indications *versus* contraindications

Of the persons who were vaccinated for the first time (Table 28), one was receiving a high dose of cortisone as sub

From the Department of Obstetrics and Gynecology Karolinska sjukhuset Stockholm Sweden

Smallpox Vaccination during Pregnancy

LARS ENGSTROM

Introduction

During the smallpox outbreak in Stockholm in the spring of 1963 the question of vaccination during pregnancy had to be considered Although mass vaccination was not adopted certain categories of pregnant women needed to be vaccinated such as nursing staff, hospitalized patients with complications of pregnancy, and persons wishing to travel abroad

The Board of Health s circular 34/1961 which is an addendum to circular 104 1958 states "Smallpox vaccination should not be done during the first three months of pregnancy This applies both to primary vaccination and revaccination since vaccination during this period involves some risk of damage to the foetus During the 1963 epidemic the Board of Health issued the following directives Pregnant women should be vaccinated irrespective of the period of gestation insofar as vaccination is recommended by the public health authorities After the end of the epidemic the Board of Health issued circular 111/1963 which stated The incidence of prenatal vaccinia in children of mothers vaccinated during later pregnancy would appear to be very low Even if the risk of serious intrauterine vaccinia virus infections is very slight it has nevertheless been considered to warrant extension of the contraindication to smallpox vaccination to cover the entire period of pregnancy In accordance herewith the Board of Health recommends that pregnant women should not be vaccinated against smallpox, particularly if they have not previously been vaccinated

If a pregnant woman contracts smallpox the prognosis is poor both for mother and child A report from Madras by Rao et al (1963) shows a high *incidence of premature births and high* mortality among foeti and mothers The maternal death rate was higher than for non pregnant women or men This implies that pregnant women should be vaccinated during a smallpox epidemic or if there is reason to expect that they may be exposed to a risk of infection

Smallpox vaccination during pregnancy however is considered to entail a risk of abortion or foetal vaccinia After the smallpox epidemic in Scotland MacArthur (1952) reported the outcome of 203 pregnancies in which the mother was vaccinated during pregnancy Of the 34 women vaccinated between the 4th and 12th week of pregnancy 10 had abortions 5 gave birth to stillborn children, 1 had a malformed child and 1 a premature The abnormity rate was thus 50 per cent

that vaccination might give rise to complications About half of the patients were vaccinated After this screening the incidence of complications was definitively higher than in the general population during the same epidemic (cf page 99) Considering the situation of the vaccinees and the general epidemiological situation the reactions can however not be regarded as objectionable

TABLE 30 Distribution of nationalities in the material

	No of cases
Nationalities	
Finnish	19
German	10
Norwegian	1
Danish	2
Greek	1
English	1
Total	34
Swedish	136
Total	*170*

Twelve of the 170 women had not previously been vaccinated against smallpox in two cases because of eczema in infancy and the remainder because vaccination had never been done The times of last vaccination are shown in table 32 Of the 158 previously vaccinated the great majority had poor immunity having last been vaccinated more than 10 years previously These women had been vaccinated at pre school age and not thereafter revaccinated The success rate for the last vaccination of these 158 *previous* vaccinees was estimated anamnestically at 129 positive 12 negative and 17 uncertain (table 32)

Method and results of vaccination

Vaccination was done by the multiple pressure method and with standard vaccine made from eggs Three series of vaccine were used without demonstrable differences in take Positive results were obtained in 140 of the 170 women Twenty did not present for examina

TABLE 31 Type end frequency of pregnancy complications in the material

Vaccinated patients hospitalized for complications of pregnancy

Complication	No of cases
Threatening premature birth	9
Cystopyelitis	2
Toxaemia	13
Diabetes mellitus	2
Heart disease	3
Rh immunization	2
Benign ovarial tumour	1
Cholelithiasis	1
Total	*33*

TABLE 32 *Time and result of previous vaccination assessed anamnestically*

Smallpox vaccination status	
Previously vaccinated	158
Not previously vaccinated	12
Time since previous vaccination	
Years	*No of cases*
>10	122
9	1
8	4
7	2
6	3
5	2
4	2
3	7
2	1
1	14
	158
Result of previous vaccination Assessed anamnestically	
Positive	129
Negative	12
Don t known	17
	158

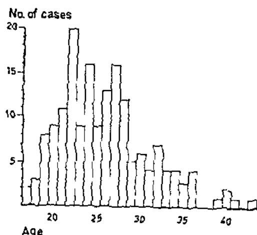


Fig 58 Age distribution

pregnancy unless there is a risk of her contracting smallpox

On account of the Board of Health recommendations and the experience reported in the literature, the Gynæcological Clinic of Karolinska Sjukhuset established a special out patient department for vaccination of pregnant women All who presented at the polyclinic were informed of the risk of miscarriage if vaccination was done before the 14th week of pregnancy During the period May 24—July 25, 1963, 170 pregnant women were vaccinated at the polyclinic

Several cases of foetal vaccinia have been reported in the literature including one case in which an unvaccinated woman contracted a quickly transient fever and cough after a child in her home had been vaccinated Two months later, in the 28th week of pregnancy, she gave birth to a child with vaccinia which later died (Lycke et al 1963)

One case of foetal vaccinia has been described also from Sweden by Lycke et al (1963) A previously unvaccinated woman was vaccinated in the 23rd week of pregnancy Five weeks later she gave birth to a child weighing 870 grammes which had skin lesions of vaccinial type and died at 8 days of age Vaccinia virus was isolated from the placenta

Although vaccinia appears to be a rare complication it does involve some risk for the foetus both during the second and the third trimester of pregnancy Considering also the risk of miscarriage and prema ri discussed in the literature in cor unction with vaccination during the fi t trimester the mother should not be vaccinated during any part of her

Material

The 170 vaccinated pregnant women were aged 17 to 43 years (fig 58) The material is a selected one from many points of view Thirty four women were foreigners chiefly Finns and Germans, who were working as hospital nurses and intended to return home during the summer holiday (table 30) The Swedish women too were mainly employed as nurses or in professions which brought them into contact with the public or they did not wish to give up a planned journey abroad Thirty three of the women were patients in hospital for treatment of pregnancy complications (table 31) A less usual cause of vaccination was fear of spread of the epidemic

Of the total material 81 were pregnant for the first time and 89 had earlier had a miscarriage or given birth to a child Of the 148 earlier pregnancies 32 had resulted in miscarriage 22 of which before and 10 after the 12th week of pregnancy

Case 1 27 year-old secund gravida wi h earl er *normal* deli ery Successfully vac c nated again t smallpox as a child and no v aga n the 12 h week of pregnancy w th pos t *e response and* wi hout compl cation Normal pregnancy up to 20th eek when she spontaneously abo ted a grossly normal *foetus* No m croscopic examina on was made An assoc at on with the smallpox vaccinat on cannot be excluded

Case 2 24 ear-old secund gra da w th earl er normal deli ery Not prev ously vac cinated against smallpox no v acc nated n 20 h week of pregnancy w th unknown result No post acc nal compl cat on Missed abort on n 28th week of p egnancy She sta ed af er the m scarriage that she had felt no fo tal mo emen s since 5 days before vac c na on Since the foetal mo ements ceased befo e vaccinat on the vaccinat on cannot ha e been a causat e factor

Ca e 3 27 year-old secund gra da w th earl er normal del ery Had been vac c nated against nfluenza n 4th week of pregnancy In 3rd mon h of pregnancy when she was unaware hat she was pregnant she had been g en gamma globul n for pre ent on of nfect ons On the same day she l ad a dull abdom nal ache and sl ght haemor rha e A p egnancy test on the next day as po e Tl e eaf er she had occas onal aches and brown aginal d scha ge A couple of weeks later n 14 h week of p egnancy) she was re acc na ed aga nst smallpox w th po e response and w hout compl ca on Nor al p egnanc unt l the 22nd week, when *he uterus ceased to grow* Missed *abortion* n tl e 30 l week. An associat on w th the smal pox accinat on cannot be excluded bu s gns of threaten ng *abort on ex s ed be* fo e she as acc nated

Of tl e 167 v omen v l o d d not abort 16? ere del vered of a l v ng child h le f e ch ldren ere stillborn The st ll born are reported on belo Of 162 l ldren born al ve 7 ere immature tl a b rtl e gl t not exceedu g 2 500 grammes The immaturity rate v as thus 4 2 per 100 live births which accords v ith the immaturity rate for the Swed sh population

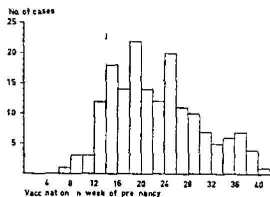
Five children v ere stillborn and three d ed n the first v eek of life

Case 4 27 year-old primogra ida vacc nated against smallpox in infancy and revaccinated in 1956 w th negat e result Vacc nated n 16th week of pregnancy w th unkno n re sult no postvacc nal compl cat on Normal pregnancy up to 40th week Abrupt on of placenta w th intrauterine death in absence of toxaemia. The child exh b ted no malforma t on or s gn of d sease No assoc at on with smallpox vaccinat on is thought to ex st.

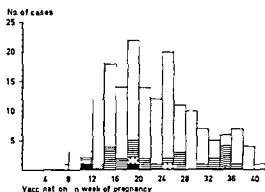
Case 5 32 year-old secund gra da w th earlier normal del ery Successfully vac cinated n infancy Unsuccessfully revac c nated in 1950 and no v th pos t e re sponse n 19 h eek of pregnancy without postvacc nal complicat on Normal preg nancy up to 35th week hen foetal mo e ments ceased After one week of sparse haemorrhage a macerated foetus was born we gh ng 1 460 grammes An assoc a on ith the smallpox va cinat on cannot be excluded

Case 6 31 year-old pr mograv da vaccina ed n nfancy and aga n successfully n 1960 Vacc na ed in 20th week of pregnancy w th *doubtful result. Repeated* vacc nat *on in* 24 h week successful No post acc nal compl ca t on Toxaem a 30 kg ncrease of we ght and hypertens *on* On del *ery at term discoloured* foetal water and intrau er ne death E en if the toxaem a may be conside ed the probable cause an assoc ation w *th smallpox* vacc na t on cannot be en rely excluded

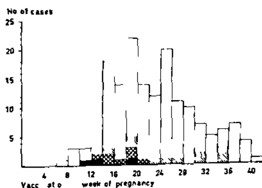
Case 7 32 year-old pr mogra da n olun tar ly sterile for some 4 years Vacc nated in infancy and again successfully one year prior to pregnancy Successfully vaccina ed in 26th week of p egnancy w thout post ac c nal compl cat on Normal p egnancy until 34 h week thereaf er sparse haemorrhage for



a



b



c

Fig 59 Time of vaccination during pregnancy (a) Duration of pregnancy in weeks (b) Foetal complications (c)

tion of the result, but reported by telephone or letter that the vaccination had been successful In 10 the response was negative Eight of them were revaccinated with the same standard vaccine, with positive result in 7 and negative in 1 The other two were vaccinated with an extra strong vaccine at another centre since previously, too, they had reacted negatively to revaccination

All vaccinees were carefully instructed to report any complications arising after the vaccination

Complications

All data relating to pregnancies, deliveries and infants were obtained from the hospital records except in two cases (deliveries in Finland and Greece), reports of which were collected from the two vaccinated women by mail A summary of the periods of pregnancy at which vaccinations were done, of the times of delivery, and of the complications encountered is given in fig 59 a, b, c In 19 cases the vaccination was done before the end of the 14th week of pregnancy at the woman s own wish after being informed of the risk of miscarriage

Two cases were reported of secondary redness of the upper arm round the vaccination site allied with a moderate rise of temperature Otherwise the mothers suffered no complication which could be related to vaccination

In 30 cases the pregnancy lasted for 38 weeks or less Abortions occurred in three cases — in one case in the 20th week of pregnancy, the other two being missed abortions in the 28th and 30th weeks

Abortions occurred in two cases among the 19 mothers vaccinated before the end of the 14th week of pregnancy (and in one of 7 mothers vaccinated before the end of the 12th week) The possibility of association with the small pox vaccination in these two cases can not be eliminated The material is too small, however for a statistical assess ment of the risk of miscarriage following vaccination in an early stage of preg nancy

The prematurity rate was 4 2 per cent, which does not differ significantly from that of the Swedish population (Allman Halso och Sjukvård 1962)

The perinatal mortality is remarkably high Stillborn in relation to total births were 3 per cent in this material as against 1 54 per cent for the population as whole in 1962 Deaths during the first week of life were 1 75 per cent of births compared with 1 11 per cent for the 1962 population The high perinatal mortality of 4 75 per cent compared with 2 65 per cent for the 1962 popula tion is due particularly to the high stillbirth rate An individual analysis of stillbirths and neonatal deaths shows that for three of the five stillbirths and one of the three neonatal deaths an as sociation with smallpox vaccination in pregnancy could not be excluded If a correction is made for these four cases, the perinatal mortality in the vaccinated material was 2 45 per cent which ac cords closely with that for the country as a whole

Apart from one case of hydrocephalus in which an association with smallpox vaccination in the 35th week of preg nancy could not be excluded, an in creased malformation rate was not found in the vaccinated material (1 8 per cent)

On account of the sudden outbreak of the epidemic and the lack of resources for prospective control of individual women delivered at different hospital in Stockholm and elsewhere inside and outside Sweden, it was not possible in any case to elucidate the significance of smallpox vaccination during pregnancy The study does show, however, that in six cases of foetal death smallpox vac cination could not be disregarded as the possible cause (3 53 per cent) In these cases the vaccinations were performed during the first, second and third tri mesters of pregnancy

The study also reveals that pregnant women in Sweden have a very poor im munity to smallpox To a large extent they have not been revaccinated since pre-school age It would appear to be an important question for the medical services to improve the immunity during the age of fertility On the other hand vaccination against smallpox should not be done during any stage of pregnancy unless there is a risk of the mother con tracting the disease

Summary

Smallpox vaccination during pregnancy was performed in 170 cases There were three abortions five children were still born and three died during the first week of life The total foetal loss was 3 53 per cent It was not possible in any case to elucidate the significance of smallpox vaccination during pregnancy but of 11 cases of foetal death an associa

a couple of days and spontaneous delivery Stillborn child which, apart from immaturity (weight 2,050 grammes), exhibited no sign of disease Association with the smallpox vaccination cannot be excluded

Case 8 29 year-old primogravida vaccinated in infancy with unknown result Successfully vaccinated in 35th week of pregnancy without postvaccinal complication She was admitted to the clinic at the time of vaccination for severe toxaemia Intrauterine death in 36th week and spontaneous delivery Birth weight 1,530 grammes The toxaemia must be considered the probable cause, an association with smallpox vaccination being unlikely

Case 9 27-year-old primogravida with juvenile diabetes Successfully vaccinated in infancy and again with positive response in 27th week of pregnancy without postvaccinal complication A caesarean operation was done in the 36th week During its first day of life the child developed respiratory distress with hyaline membranes from which death followed Birth weight 2,970 grammes Association with smallpox vaccination improbable

Case 10 27 year old secundigravida with earlier normal delivery and healthy child Vaccinated against smallpox 10 years prior to pregnancy with unknown result Successfully revaccinated in 31st week of pregnancy without postvaccinal complication Normal pregnancy Spontaneous delivery at term Birth weight 3 340 grammes The child died two days after birth Postmortem showed congenital heart disease (aortal transposition transposition of pulmonary artery and right ventricular hypertrophy) Since the growth of the foetal heart may be said to be completed by the 12th week of pregnancy (Montagu Prenatal Influences 1962 p 276) no association with smallpox vaccination can have existed

Case 11 34-year old secundigravida with previous normal delivery and healthy child Successfully vaccinated in infancy Revaccinated in 35th week of pregnancy with positive result and without postvaccinal complication Rh immunized Radiologically detected hydrocephalus Caesarean operation in 40 week of pregnancy Birth weight 3 890 grammes Child died two days later of bronchopneumonia Postmortem revealed hydrocephalus and agenesia of left kidney An association with the smallpox vaccination cannot be excluded Influenza infection during pregnancy has proved to result in an increased incidence of malformations (Coffey and Jessop 1959), particularly as regards the nervous system, such as anencephaly and hydrocephalus The possibility of an association between influenza infection during the third trimester of pregnancy and hydrocephalus of the child has been pointed out by Pleydell (1960)

No examination was made for toxoplasmosis in the mother

Malformation of the child occurred in three cases Apart from the above mentioned two cases of congenital heart disease and hydrocephalus, there was one case of bilateral hip joint dislocation Statistically the number of malformations among children in the vaccinated material was not above normal

Foetal vaccinia or scars after healed foetal vaccinia were not observed in any case

Discussion

Owing to the selective character of the material in that it incorporated chiefly foreign women, nursing staff and women hospitalized for complications of pregnancy, comparisons with the population as a whole can only be made with some caution

No significant complications due to vaccination were found among the mothers

Abortions occurred in two cases among the 19 mothers vaccinated before the end of the 14th week of pregnancy (and in one of 7 mothers vaccinated before the end of the 12th week) The possibility of association with the smallpox vaccination in these two cases can not be eliminated The material is too small however, for a statistical assessment of the risk of miscarriage following vaccination in an early stage of pregnancy

The prematurity rate was 4 2 per cent, which does not differ significantly from that of the Swedish population (Allman Halso- och Sjukvård 1962)

The perinatal mortality is remarkably high Stillborn in relation to total births were 3 per cent in this material as against 1 54 per cent for the population as whole in 1962 Deaths during the first week of life were 1 75 per cent of births compared with 1 11 per cent for the 1962 population The high perinatal mortality of 4 75 per cent compared with 2 65 per cent for the 1962 population is due particularly to the high stillbirth rate An individual analysis of stillbirths and neonatal deaths shows that for three of the five stillbirths and one of the three neonatal deaths an association with smallpox vaccination in pregnancy could not be excluded If a correction is made for these four cases the perinatal mortality in the vaccinated material was 2 45 per cent, which accords closely with that for the country as a whole

Apart from one case of hydrocephalus in which an association with smallpox vaccination in the 35th week of pregnancy could not be excluded, an increased malformation rate was not found in the vaccinated material (1 8 per cent)

On account of the sudden outbreak of the epidemic and the lack of resources for prospective control of individual women delivered at different hospitals in Stockholm and elsewhere inside and outside Sweden it was not possible in any case to elucidate the significance of smallpox vaccination during pregnancy The study does show, however, that in six cases of foetal death smallpox vaccination could not be disregarded as the possible cause (3 53 per cent) In these cases the vaccinations were performed during the first, second and third trimesters of pregnancy

The study also reveals that pregnant women in Sweden have a very poor immunity to smallpox. To a large extent they have not been revaccinated since preschool age It would appear to be an important question for the medical services to improve the immunity during the age of fertility On the other hand vaccination against smallpox should not be done during any stage of pregnancy unless there is a risk of the mother contracting the disease

Summary

Smallpox vaccination during pregnancy was performed in 170 cases There were three abortions five children were stillborn and three died during the first week of life The total foetal loss was 3 53 per cent It was not possible in any case to elucidate the significance of smallpox vaccination during pregnancy but of 11 cases of foetal death an associa

tion with the smallpox vaccination could not be excluded in six Foetal vaccinia was not observed in any case There was no increase of prematurity rate Apart from one case of hydrocephalus, the smallpox vaccination could not be related to any increase in the incidence of malformations

Vaccination against smallpox during pregnancy should not be done unless there is a risk of smallpox infection for the mother Since Swedish women have proved to have poor immunity to smallpox, revaccinations should be performed during the age of fertility

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From the Department of Dermatology (Head Professor Sven Hellerström) and the Department of Clinical Bacteriology (Head Associate Professor Hans Ericsson) Karolinska Sjukhuset, Stockholm 60

The Assessment, and the Vaccination, of Patients with Cutaneous Disorders

GUNNEL ERIKSSON and MARGIT FORSBECK

During the 1963 outbreak of smallpox in Stockholm we were requested to make a dermatological assessment of the suitability for vaccination of persons with cutaneous or allergic disorders who had sought advice on this matter at the Bacteriology Laboratory, Karolinska Sjukhuset The majority of these persons believed that by using a special vaccine or immune serum globulin we could guarantee uncomplicated primary vaccination They had been encouraged in this belief by the press radio, television and also to some extent by members of the medical profession

All who have treated cases of eczema vaccinatum know how serious this condition is During the period 1954 to 1963 seven cases of generalised vaccinia were registered at the Department of Dermatology Karolinska Sjukhuset (1) Six were classified as eczema vaccinatum one as vaccinia inoculata All had atopic dermatitis (Prurigo Besnier) Two of the patients had received routine smallpox vaccination and in three cases contamination had occurred from recently vaccinated relatives One patient had used a contaminated bath Four of the seven patients had not previously been vaccinated against smallpox

The first published description of eczema vaccinatum was presented by Martin (2) in 1882 Small numbers of cases have subsequently been reported from many countries (3—11) The patients were usually less than five years old and had a history of eczema Contamination from recently vaccinated contacts was a much more common evoking factor than direct vaccination. Some writers, e g Naseman (12) and Chobot (13) expressed the opinion that persons who have had atopic dermatitis should not be vaccinated until the cutaneous lesions have been healed for at least one year Others, such as Glaser (14) permitted vaccination after a shorter period of healing which could be as little as three weeks It is relevant in this connection that Paschen (15) reported a case of eczema vaccinatum occurring after the atopic dermatitis had been apparently healed for 1 year Lindahl and Espmark (16) published a report on a small series of complication free vaccinations on persons with atopic dermatitis They used a weaker vaccine than that commonly administered and they gave immune serum globulin when the vaccination papule began to appear

TABLE 33 Applicants for smallpox vaccination reviewed on grounds of dermatological suitability

	Vaccinated				Not vaccinated
	Number vaccinated	Follow up visits	Follow up by telephone or letter only	No follow up	
Primary vaccination	209	490	6	7	208
Revaccination	793	898	229	38	265
Total	*1002*	*1388*	*235*	*45*	*473*

In other types of eczema the risk of virus dissemination after smallpox vaccination is generally considered to be small, but it is recommended that the vaccine should not be given while the lesions are in an acute exudative phase The non specific irritation involved in vaccination can lead to a flare-up of cutaneous disorders such as psoriasis (17) A few cases of postvaccinial complications have been reported in eczema seborrhoicum, contact dermatitis, impetigo contagiosa, acne vulgaris, ichthyosis, Darier's dyskeratosis, etc

The fact that the Swedish Royal Medical Board permits exemption from smallpox vaccination in some cases of eczema (18) constitutes further evidence of the potential risks involved in such vaccination

During the recent outbreak of smallpox in Stockholm, therefore, the obvious advisability of cautious use of vaccine had to be considered against the widespread public demand for vaccination Our work was done on an out patient basis When the outbreak began we had at our disposal 100 doses of immune serum globulin against vaccinia (prepared at the National Bacteriology Laboratory), each dose consisting of 5 ml Of the effect of this substance we knew little Our aim was to avoid, as far as possible, serious complications such as eczema vaccinatum, a condition which can be as serious as smallpox itself This was particularly important as long as the outbreak of smallpox was confined to the immediate contacts of the cases (the "inner circle")

Some of the persons who were referred for our assessment had had contact with cases of smallpox Others had occupational indications for vaccination (police, hospital staff, etc) A third group consisted of persons whose plans for the current summer included community living in camps, etc , or who had planned foreign travel

In recording medical histories special attention was paid to the duration and intensity of cutaneous disorders We were particularly restrictive when the applicants had not previously been vaccinated or when a long time had elapsed since the foregoing vaccination Infants with atopic eczema, and in particular boys from the pre school and primary

TABLE 31 Persons advised against smallpox vaccination after dermatological assessment

Diagnosis	Primary vaccination	Revaccination	*Total*
Atopic dermatitis			
Healed for more than one month	3	2	5
Lichenification erythema moderate excoriation	21	22	43
Extensive lesions	126	58	184
Ichthyosis	1	2	3
Urticaria / *Allergic rhinitis* / *Bronchial asthma*	3	4	7
Psoriasis	3	2	-
Eczema			
Seborrhoic / Occupational / Hypostatic / Localised neurodermatitis / Neurotic excoriations / Mycosis	4	37	41
Acne			
Acne vulgaris / Acne rosacea / Furunculosis	—	3	3
Lichen ruber planus	—	2	2
Healed eczema (not atopic dermatitis)	—	—	—
Miscellaneous (e g dermatitis herpetiformis, herpes simplex	1	5	6
Other reasons for advising against vaccination (cardiovascular disease advanced age social reasons etc)			17x
Total	162	137	4 3

school age groups were judged to run considerably greater risk than older persons The *type* of dermatological manifestations in the older persons was considered to be less important as a contra indication to vaccination We did not advise vaccination unless we could assume from personal contact with the patient or his relatives that our directions would be satisfactorily followed

Vaccine prepared by the yolk sac culture method was used unless there was known or suspected hypersensitivity to egg in which case calf lymph vaccine was used Vaccination was performed on a site free from lesions and which could

TABLE 33 Applicants for smallpox vaccination reviewed on grounds of dermatological suitability

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In recording medical histories special attention was paid to the duration and intensity of cutaneous disorders We were particularly restrictive when the ap plicants had not previously been vaccinated or when a long time had elapsed since the foregoing vaccination Infants with atopic eczema, and *in particular* boys from the pre-school and primary

TABLE 34 Persons advised against smallpox vaccination after dermatological assessment

Diagnosis	Primary vaccination	Revaccination	*Total*
Atopic dermatitis			
Healed for more than one month	3	2	*5*
Lichenification erythema moderate excoriation	21	22	*43*
Extensive lesions	126	58	*184*
Ichthyosis	1	2	*3*
Urticaria / *Allergic rhinitis* / *Bronchial asthma*	3	4	*7*
Psoriasis	3	2	[illegible]
Eczema — Seborrhoic, Occupational, Hypostatic, Localised neurodermatitis, Neurotic excoriations, Mycosis	4	37	*41*
Acne — Acne vulgaris, Acne rosacea, Furunculosis	–	3	*3*
Lichen ruber planus	–	2	*2*
Healed eczema (not atopic dermatitis)	–	–	–
Misllellaneous (e g dermatitis herpetiformis herpes simplex	1	5	*6*
Other reasons for advising against vaccination (cardiovascular disease advanced age social reasons etc)			*1/4*
Total	*162*	*137*	*4/3*

school age groups were judged to run considerably greater risk than older persons The *type* of dermatological manifestations in the older persons was considered to be less important as a contra indication to vaccination We did not advise vaccination unless we could assume from personal contact with the patient or his relatives that our directions would be satisfactorily followed

Vaccine prepared by the yolk sac culture method was used unless there was known or suspected hypersensitivity to egg in which case calf lymph vaccine was used Vaccination was performed on a site free from lesions and which could

TABLE 33 Applicants for smallpox vaccination reviewed *on grounds of dermatological suitability*

	Vaccinated				*Not vaccinated*
	Number vaccinated	Follow up visits	Follow up by telephone or letter *only*	No follow up	
Primary vaccination	209	490	6	7	208
Revaccination	*793*	*898*	229	38	265
Total	*1002*	*1388*	*235*	*45*	*473*

In *other types of* eczema the risk of *virus* dissemination after smallpox vac*cination is generally considered to be* small, but it is recommended that the vaccine should not be given while the lesions are in an acute exudative phase *The non specific irritation involved in* vaccination can lead to a flare up of cutaneous disorders such as psoriasis *(17) A few cases of postvaccinial* complications have been reported in eczema seborrhoicum, contact dermatitis, impetigo contagiosa, acne vulgaris, ichthyosis, Darier s dyskeratosis, etc

The fact that the Swedish Royal Med*ical Board permits exemption from small*pox vaccination in some cases of eczema (18) constitutes further evidence of the potential risks involved in such vaccina*tion*

During the recent outbreak of smallpox in Stockholm, therefore, the obvious advisability of cautious use of vaccine *had to be* considered against the wide spread public demand for vaccination Our work was done on an out patient basis When the outbreak began we had at our disposal 100 doses of immune serum globulin against vaccinia (prepared at the National Bacteriology Laboratory), each dose consisting of 5 ml *Of the effect of this substance we knew* little *Our aim was to avoid, as far as* possible, serious complications such as eczema vaccinatum, a condition which can be as serious as smallpox itself This was particularly important as long as the outbreak of smallpox was confined to the immediate contacts of the cases (the "inner circle")

Some of the persons who were referred for our assessment had had *contact* with cases of smallpox Others had occupational indications for vaccination *(police, hospital* staff, etc) A third group consisted of persons whose plans for the current summer included community living in camps, etc , or who had planned foreign travel

In recording medical histories special attention was paid to the duration and intensity *of cutaneous disorders We were* particularly restrictive when the applicants had not previously been vaccinated or when a long time *had elapsed* since the foregoing vaccination *Infants* with atopic eczema, and in particular boys from the pre school and primary

TABLE 34 Persons advised against smallpox vaccination after dermatological assessment

Diagnosis	Primary vaccination	Revaccination	*Total*
Atopic dermatitis			
Healed for more than one month	3	2	*5*
Lichenification erythema moderate excoriation	21	22	*43*
Extensive lesions	126	58	*184*
Ichthyosis	1	2	*3*
Urticaria / *Allergic rhinitis* / *Bronchial asthma*	3	4	*7*
Psoriasis	3	2	*5*
Eczema: Seborrhoic, Occupational, Hypostatic, Localised neurodermatitis, Neurotic excoriations, Mycosis	4	37	*41*
Acne: Acne vulgaris, Acne rosacea, Furunculosis	—	3	*3*
Lichen ruber planus	—	2	*2*
Healed eczema (not atopic dermatitis)	—	—	—
Miscellaneous (e g dermatitis herpetiformis herpes simplex	1	5	*6*
Other reasons for advising against vaccination (cardiovascular disease advanced age social reasons etc)			*174*
Total	*162*	*137*	[illegible]

school age groups, were judged to run considerably greater risk than older persons The *type* of dermatological manifestations in the older persons was considered to be less important as a contraindication to vaccination We did not advise vaccination unless we could assume from personal contact with the patient or his relatives that our directions would be satisfactorily followed

Vaccine prepared by the yolk sac culture method was used unless there was known or suspected hypersensitivity to egg, in which case calf lymph vaccine was used Vaccination was performed on a site free from lesions and which could

TABLE 35 Vaccinated persons according to diagnosis and age

Diagnosis	< 1 yr P	< 1 yr R	1—5 yrs P	1—5 yrs R	6—13 yrs P	6—13 yrs R
Atopic dermatitis						
Healed for more than one month	1		14	9	21	14
Lichenification erythema moderate excoriation	1		8	3	20	22
Extensive lesions	1		2	1	1	
Ichthyosis						
Urticaria / *Allergic rhinitis* / *Bronchial asthma*						4
Psoriasis			1		2	
Eczema						
Seborrhoic / Occupational / Hypostatic / Localised neurodermatitis / Neurotic excoriations / Mycosis			1	1	2	3
Acne						
Acne vulgaris / Acne rosacea / Furunculosis						
Lichen ruber planus						
Healed eczema (not atopic dermatitis)			1		2	2
Miscellaneous (e.g. dermatitis herpetiformis, herpes simplex)			1		5	
Total	3		8	7	51	45

P primary vaccination R revaccination

easily be covered until the crust had dried As a rule the distal part of the upper arm was chosen When the vaccine had dried the site was covered with a fairly large compress which was fastened by surgical tape (micropore tape Minnesota Mining and Manufacturing Company) A firm covering of tubular gauze was then drawn over the whole upper arm This dressing had no unfavourable effects on the local vaccination reaction or on the surrounding skin

The patients were instructed to return for examination on the day that the vesicle was expected to appear or earlier if there was any worsening of the basic disorder Observation was then continued daily if necessary until the crust was dry

14—20 yrs		21—40 yrs		41—60 yrs		>60 yrs		*Total*		
P	R	P	R	P	R	P	R	P	R	P+R
23	20	14	25	1	1			74	62	*136*
22	41	13	26	1	4			65	96	*161*
	1	2	3		1			6	6	*12*
	3	1	1	1	1			2	5	*7*
3	10	3	2		9			6	25	*31*
3	13	6	55	6	57		3	18	128	*146*
5	31	8	150	8	170		12	24	367	*391*
	16		10	1	2			1	28	*29*
	1		2		2				5	*5*
2	5		8	1	22		3	6	40	*46*
2	4		12		14	1	1	7	31	*38*
[illegible]	*145*	*47*	*294*	*19*	*283*	*1*	*19*	*209*	*793*	*1002*

As the clinical material was selected it was not suitable for statistical treatment with respect to hypotheses such as that smallpox vaccination involves definite risk in subjects with active eczema or that immune serum globulin has a special action in vaccination of persons with cutaneous or allergic disorders Consequently the following review must be purely descriptive.

The total number of cases assessed by us because of diseases of the skin or allergic disorders was 1475 (table 33) Of these 1002 were accepted for vaccination and 473 rejected All but 45 of the vaccinated persons were followed up however, in 235 cases this was confined to enquiries by letter or telephone The total number of repeat visits was 1388 The vaccination was read as

TABLE 35 Vaccinated persons according to diagnosis and age

Diagnosis	<1 yr		1—5 yrs		6—13 yrs	
	P	R	P	R	P	R
Atopic dermatitis						
Healed for more than one month	1		14	2	21	14
Lichenification, erythema, moderate excoriation	1		8	3	20	22
Extensive lesions	1		2	1	1	
Ichthyosis						
Urticaria						
Allergic rhinitis						4
Bronchial asthma						
Psoriasis			1		2	
Eczema						
Seborrhoic						
Occupational						
Hypostatic			1	1	2	3
Localised neurodermatitis						
Neurotic excoriations						
Mycosis						
Acne						
Acne vulgaris						
Acne rosacea						
Furunculosis						
Lichen ruber planus						
Healed eczema (not atopic dermatitis)			1		2	2
Miscellaneous (e g dermatitis herpetiformis herpes simplex)			1		3	
Total	3		28	7	51	45

P primary vaccination R revaccination

easily be covered until the crust had dried As a rule the distal part of the upper arm was chosen When the vaccine had dried the site was covered with a fairly large compress which was fastened by surgical tape (micropore tape, Minnesota Mining and Manufacturing Company) A firm covering of tubular gauze was then drawn over the whole upper arm This dressing had no unfavourable effects on the local vaccination reaction or on the surrounding skin

The patients were instructed to return for examination on the day that the vesicle was expected to appear, or earlier if there was any worsening of the basic disorder Observation was then continued daily if necessary, until the crust was dry

TABLE 37 Satellite or secondary vaccinal lesions

Diagnosis	Satellite lesions	Secondary lesions	Vaccinia
Atopic dermatitis			
Heald for more than one month	14	1	
Lichenification erythema moderate excoriation	12	4	
Extensive lesions	1		
Ichthyosis	2	2	
Urticaria			
Allergic rhinitis			
Bronchial asthma			
Psoriasis		1	
Eczema			
Seborrhoic	3		
Occupational			
Hypostatic			
Localised neurodermatiti			
Neurotic excoriations			
Mycosis			
Acne			
Acne vulgaris			
Acne rosacea			
Furunculosis			
Lichen ruber planus			
Healed eczema (not atopic dermatitis)	1		
Miscellaneous (e g dermatitis herpetiformis herpes simplex)	2	1	
Total	*35*	*9*	*0*

cases) moderately severe, active manifestations (161) and extensive lesions (12) 146 psoriatic patients were vaccinated The largest group comprised 391 persons with assorted types of eczema many of them with active lesions when vaccination was performed

The age distribution of the series was in conformity with the distribution of the skin diseases Thus atopic dermatitis occurred predominantly among younger patients and the eczematous persons tended to be older

None of the patients who were followed up developed eczema vaccinatum A 60 year old patient with eczema of the hands was admitted to hospital because of suspected encephalitis Electroencephalograms and the cerebrospinal fluid were then found to be normal Two more of the vaccinated subjects complained of protracted headaches

TABLE 36 Worsening of initial disorder following smallpox vaccination

Diagnosis	Primary vaccination	Revaccination	*Total*
Atopic dermatitis			
Healed for more than one month	9/74	5/62	*14/136*
Lichenification, erythema, moderate excoriation	10	11	*21/161*
Extensive lesions	1		*1*
Ichthyosis			
Urticaria }			
Allergic rhinitis }	1	4	*5*
Bronchial asthma }			
Psoriasis	1	5	*6*
Eczema			
Seborrhoic }			
Occupational }			
Hypostatic }			
Localised neurodermatitis }	3	11	*14/391*
Neurotic excoriations }			
Mycosis }			
Acne			
Acne vulgaris }			
Acne rosacea }			
Furunculosis }			
Lichen ruber planus		1	*1*
Healed eczema (not atopic dermatitis)	1		*1*
Miscellaneous (e g dermatitis herpetiformis herpas simplex)		1	*1*
Total	26	38	*64/1002*

successful in 850 cases (primary vaccination 200, revaccination 650) Calf lymph vaccine was used in 138 cases

In 174 cases the decision to advise against vaccination was based upon reasons other than the actual disorder of the skin (e g cardiovascular disease, advanced age, social reasons, etc)

Among the 299 persons who were advised against vaccination because of diseases of the skin the largest group had not previously been vaccinated Most of these patients had atopic dermatitis (table 34)

Table 35 reviews the vaccinated patients with respect to diagnosis, age and whether or not the vaccination was primary 309 patients with atopic dermatitis were vaccinated They were divided into three sub groups more than a month of freedom from symptoms immediately prior to vaccination (136

TABLE 37 Satellite or secondary vaccinal lesions

Diagnosis	Satellite lesions	Secondary lesions	Vaccinia
Atopic dermatitis			
Heald for more than one month	14	1	
Lichenification erythema, moderate excoriation	12	4	
Extensive lesions	1		
Ichthyosis	2	2	
Urticaria			
Allergic rhinitis			
Bronchial asthma			
Psoriasis		1	
Eczema			
Seborrhoic	3		
Occupational			
Hypostatic			
Localised neurodermatitis			
Neurotic excoriations			
Mycosis			
Acne			
Acne vulgaris			
Acne rosacea			
Furunculosis			
Lichen ruber planus			
Healed eczema (not atopic dermatitis)	1		
Miscellaneous (e g dermatitis herpetiformis herpes simplex)	2	1	
Total	*35*	*9*	*0*

cases) moderately severe, active manifestations (161), and extensive lesions (12) 146 psoriatic patients were vaccinated The largest group comprised 391 persons with assorted types of eczema, many of them with active lesions when vaccination was performed

The age distribution of the series was in conformity with the distribution of the skin diseases Thus atopic dermatitis occurred predominantly among younger patients and the eczematous persons tended to be older

None of the patients who were followed up developed eczema vaccinatum A 60 year old patient with eczema of the hands was admitted to hospital because of suspected encephalitis Electroencephalograms and the cerebrospinal fluid were then found to be normal Two more of the vaccinated subjects complained of protracted headaches

TABLE 36 Worsening of initial disorder following smallpox vaccination

Diagnosis	Primary vaccination	Revaccination	*Total*
Atopic dermatitis			
Healed for more than one month	9/74	5/62	*14/136*
Lichenification, erythema, moderate excoriation	10	11	*21/161*
Extensive lesions	1		*1*
Ichthyosis			
Urticaria / *Allergic rhinitis* / *Bronchial asthma*	1	4	*5*
Psoriasis	1	5	*6*
Eczema			
Seborrhoic / Occupational / Hypostatic / Localised neurodermatitis / Neurotic excoriations / Mycosis	3	11	*14/391*
Acne			
Acne vulgaris / Acne rosacea / Furunculosis			
Lichen ruber planus		1	*1*
Healed eczema (not atopic dermatitis)	1		*1*
Miscellaneous (e g dermatitis herpetiformis, herpas simplex)		1	*1*
Total	26	38	*64/1002*

successful in 850 cases (primary vaccination 200, revaccination 650) Calf lymph vaccine was used in 138 cases

In 174 cases the decision to advise against vaccination was based upon reasons other than the actual disorder of the skin (e g cardiovascular disease, advanced age, social reasons, etc)

Among the 299 persons who were advised against vaccination because of diseases of the skin the largest group had not previously been vaccinated Most of these patients had atopic dermatitis (table 34)

Table 35 reviews the vaccinated patients with respect to diagnosis, age and whether or not the vaccination was primary 309 patients with atopic dermatitis were vaccinated They were divided into three sub groups, more than a month of freedom from symptoms immediately prior to vaccination (136

TABLE 39 Administration of immune serum globulin effect and complications

Diagnosis	1 dose immune serum globulin	Apparantly good clinical effect	Repeated injections	Complications
Atopic dermatitis				
Healed for more than one month	20	12	1	
Lichenification erythema moderate excoriation	30	10	1	
Extensive lesions	7	5		
Ichthyosis	2	1		
Urticaria *Allergic rhinitis* *Bronchial asthma*	1			1
Psoriasis	10	5	1	
Eczema Seborrhoic Occupational Hypostatic Localised neurodermatitis Neurotic excoriations Mycosis	22	6	3	
Acne Acne vulgaris Acne rosacea Furuculosis	1			1
Lichen ruber planus				
Healed eczema (not atopic dermatitis)	1			
Miscellaneous (e g dermatitis herpetiformis, herpes simplex)	4	2		1
Total	*98*	*41*	*6*	*3*

Each injected dose – 5 ml specific immune serum globulin against vaccinia (State Bacteriology Laboratory) Complications – papular rash.

duration erysipelas like erythema and necrosis at the site of vaccination occurred predominantly among older persons whose dermatoses presented a potentially minor risk in vaccination

Exanthematous complications were few (table 38) Such complications are described by Rajka and Nilzen (p 158) There were five cases of maculopapular exanthema Only one patient developed

TABLE 38 Exanthematous and urticarial complications following smallpox vaccination

Diagnosis	*Maculo papular rash*	Pityriasis rosea	*Urticaria round vaccinal lesion*	Papular rash after immune serum globulin
Atopic dermatitis				
Healed for more than one month				
Lichenification, erythema, moderate excoriation	1	1		
Extensive lesions				
Ichthyosis	1			
Urticaria				
Allergic rhinitis				1
Bronchial asthma				
Psoriasis	1		1	
Eczema				
Seborrhoic				
Occupational				
Hypostatic				
Localised neurodermatitis	2		1	
Neurotic excoriations				
Mycosis				
Acne				
Acne vulgaris				
Acne rosacea				1
Furunculosis				
Lichen ruber planus				
Healed eczema (not atopic dermatitis)				
Miscellaneous (e g dermatitis herpetiformis, herpes simplex)				1
Total	5	1	2	3

Another reported cardiac symptoms, but she failed to attend an arranged cardiac examination

In 64 cases flare-up of the basic cutaneous disorder occurred in association with vaccination (table 36) 36 of these patients had atopic dermatitis, which in 14 cases had been quiescent for at least a month prior to vaccination

In addition to flare-up, we feared the appearance of satellite or secondary vaccinial lesions which could portend eczema vaccinatum (table 37) This complication occurred in 35 cases, 27 of which had atopic dermatitis Such complications occurred in 4 of the 7 patients with ichthyosis and were more extensive than in the other cases *In*

9 Kober G Dtsch Gesundh Wes 13 933 1958
10 Fromer J Greenspan J and Perrin E Lahey Clin. Bull 10 200 1958
11 Reynolds E Lancet 2 684 1960
12 Nasemann T Hdb d. Haut u Geschl Kr v J Jadassohn Ergänzungswerk II 2 S 173 1961
13 Chobot R Pediatric allergy New York 1951
14 Glaser J Allergy in Childhood Springfield 1956
15 Paschen E Hdb d. Haut u Geschl Kr v J Jadassohn Bd 11 S 243, 1932
16 Lindahl J och Espmark Å Variolavaccination av eksempatienter Nord med. T 69 687 1963
17 Strandberg J Nord med T 1 91 1929
18 Medicinalstyrelsens författning no 62 21 4 1930

pityriasis rosea in association with vaccination Two had urticaria around the vaccinal lesion

The indications for administration of immune serum globulin were variable, as was the time of administration The most common indications for its use in primary vaccination of persons with atopic dermatitis were worsening of the eczema or appearance of satellite or secondary vaccinal vesicles Severe general malaise and extensive induration around the vaccination site were likewise indications for immune serum globulin Altogether 98 patients, 57 of whom had atopic dermatitis, received immune serum globulin (table 39) In 41 cases the injection was followed by rapid regression of the vaccination reaction and of the basic cutaneous disorder Six patients received more than one injection of immune serum globulin, but they seemed to respond neither to the initial injection nor to repeat administration In three cases a papular rash appeared after injection of immune serum globulin, aut it subsided after two or three days bnd there were no other symptoms

Summary

The suitability for smallpox vaccination of 1475 persons with cutaneous disorders was assessed Vaccination was performed in 1002 cases (primary vaccination in 209 cases and revaccination in 793) In 299 cases vaccination was refused on dermatological grounds, and in 174 cases on other grounds

The patients selected for vaccination were carefully studied, with particular reference to the duration and intensity of their disease An occlusive dressing was applied to the site of the vaccination and the patients were closely observed There were no cases of eczema vaccinatum The number and type of other complications, however, underline the necessity for caution in vaccination of persons with atopic dermatitis and active eczema

Unvaccinated eczematous persons constitute a risk group in outbreaks of smallpox Their disease may be regarded as a contraindication to vaccination of healthy family members Foreign travel may thus be restricted For all these reasons it would be valuable if vaccination of eczematous patients could be performed on a scale sufficient to permit statistical analysis and to provide answers to the following hypothetical questions What are the risks of vaccinating patients with atopic dermatitis? Is immune serum globulin of value? If so, at what time should immune serum globulin be given?

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8 REYNOLDS, A and JOOS, H Pediatrics *22* 259 1958

Table 40

	Cases of post vaccinal dermatosis recently vaccinated		Controls not recently vaccinated		Controls recently vaccinated conscripts		Controls frequently vaccinated	
	Immediate	Delayed	Immediate	Delayed	Immediate	Delayed	Immediate	Delayed
I Nonreactive concentrations								
Vaccinia virus 1/400	0	0	0	0				
Preparation I 1/20	0–(+)	0–+	0	0	+	+		
Preparation II 1/100	0–(+)	+	0	0	+	+		
Yolk sac extract 1/10	0–(+)	0–+	0	0				
Calf kidney control 1/10			0	0				
Egg tissue control	0	0	0	0	0	0–(+)		
II Marginal reactive concentrations								
Vaccinia virus 1/60	0	0–+	0–(+)	0–(+)	(+)	(+)	0	(+)
Preparation I 1/2	+	+	0–(+)	+	+	+–++	+	+–++
Preparation II 1/10	(+)	+–++	0–(+)	0–+	+	+–++	0–+	+–++
Calf kidney control	(+)	0	(+)	0	(+)	0	(+)	0

Preparation I = Rapidly centrifuged fluid from vaccinia infected culture of calf kidney

Preparation II = Purified and concentrated soluble antigen prepared from vaccinia infected culture of calf kidney

Including tests of our as well as of the cases of Norén et al

Negative reactions 0 Positive reactions (+) + ++

Mechanism

As regards the relationship between smallpox revaccination and dermatologic complications two groups are distinguishable

1 In some cases vaccination is only a trigger factor in the occurrence or recurrence of urticaria, eczema etc, or it is one of the agents producing a multiaetiologic phenomenon such as erythema multiforme

2 In cases of postvaccinal eruption of erysipelas, papular or parapsoriasis type, a causal connection with vaccina-

From the Department of Allergy (Head Professor Å Nilzen) Karolinska Sjukhuset, Stockholm Sweden

Postvaccinal Dermatoses

Georg Rajka and Åke Nilzen

Twenty-seven cases of exanthema following smallpox vaccination were treated by us This series does not include inoculation cases or secondary vaccination lesions

The 27 cases could be classified as follows

1 *Erysipelas-like, severe local reactions* (erythema and oedema involving large areas of the inoculated arm) developed in five patients Two had not previously been vaccinated and in two others there was a long interval between the foregoing and the present vaccination In the fifth case several previous vaccinations had failed to take

2 *Reactivation of cutaneous disorder* occurred in four cases, constituting recurrence of urticaria in three In one of these patients the urticaria commenced during the postvaccinal pyrexia This patient had a history of urticaria in response to external heat or to elevated body temperature Seborrhoeic eczema appeared after vaccination in a patient who was already known to have seborrhoea All these reactions were analogous with reactivation of other disorders, e g encephalitis, following smallpox vaccination

3 *Erythema multiforme type rash* appeared after vaccination in four cases In one case erythema nodosum simultaneously appeared and in another case there was concomitant aphthae The interval between vaccination and occurrence of the rash varied In one case it appeared after primary vaccination and in the other cases after revaccination Postvaccinal erythema multiforme has been described by several writers The causal mechanism is not yet clear, but it is conceivable that vaccinia virus is responsible for the activation of the symptoms A statistical analysis of the seasonal incidence of erythema multiforme would help to elucidate the question of coincidence or causal connection

4 *Papular rashes* comprises morbilliform, rubeoliform, follicular, lichenoid papulo urticarial eruptions and pityriasis rosea arising after smallpox vaccination There were eight cases in the group and the symptoms appeared on average ten days after vaccination

5 *Parapsoriasis-like exanthema* followed vaccination in five cases and affected mainly the extremities Histologic studies showed somewhat thin epithelium, focal necrosis of the cutis and distinct vascular changes with little cellular infiltration

6 *Coincidental rash* may be defined as an eruption that appears in association with smallpox vaccination, but a causal connection between the two can be excluded In our case the rash consisted o facial impetigo

TABLE 40*

	Cases of post vaccinal dermatosis, recently vaccinated		Controls not recently vaccinated		Controls, recently vaccinated conscripts		Controls frequently vaccinated	
	Immediate	Delayed	Immediate	Delayed	Immediate	Delayed	Immediate	Delayed
I Nonreactive concentrations								
Vaccinia virus 1/400	0	0	0	0				
Preparation I 1/20	0-(+)	0-+	0	0	+	+		
Preparation II 1/100	0-(+)	+	0	0	+	+		
Yolk sac extract 1/10	0 (+)	0-+	0	0				
Calf kidney control 1/10			0	0				
Egg tissue control	0	0	0	0	0	0-(+)		
II Marginal reactive concentrations								
Vaccinia virus 1/60	0	0-+	0-(+)	0-(+)	(+)	(+)	0	(+)
Preparation I 1 2	+	+	0-(+)	+	+	+ ++	+	+-++
Preparation II 1 10	(+)	+ ++	0-(+)	0-+	+	+-++	0-+	+-++
Calf kidney control	(+)	0	(+)	0	(+)	0	(+)	0

Preparation I – Rapidly centrifuged fluid from vaccinia infected culture of calf kidney

Preparation II – Purified and concentrated soluble antigen prepared from vaccinia infected culture of calf kidney

* Including tests of our as well as of the cases of Noren et al

Negative reactions 0 Positive reactions (+) + ++

Mechanism

As regards the relationship between smallpox revaccination and dermatologic complications two groups are distinguishable

1 In some cases vaccination is only a trigger factor in the occurrence or recurrence of urticaria, eczema, etc, or it is one of the agents producing a multiaetiologic phenomenon such as erythema multiforme

2 In cases of postvaccinal eruption of erysipelas, papular or parapsoriasis type a causal connection with vaccina-

tion seems to be more probable At present, however, it is not possible to state that this is the whole explanation

Aetiology

In the aetiology of such postvaccinal dermatoses we have to consider the role of exogenous factors and that of the vaccinated individual

I *Exogenous factors (vaccine)*

The egg component Most of these twenty-seven patients had received Swedish vaccine prepared by the yolk-sac culture method None of them had a history of hypersensitivity to egg, but two had a history of digestive intolerance because of cholecystopathy Intracutaneous tests with extracts of egg white and egg yolk were negative in all 27 cases

Allergy to complete vaccines Allergy to yolk sac or calf lymph vaccine could not be demonstrated by skin tests in any patient The tests were made by scarification None of the patients gave an immediate reaction Nor were there any delayed reactions, except in three patients who showed typical "reactions of immunity" The tests were made three to five weeks after smallpox vaccination

Allergy to vaccine fractions Nine of our twenty-seven patients were tested with the vaccine fractions described by Noren et al (see theirs paper in this supplement) The frequency of positive reactions was not higher than in control subjects (table 40) This applied both to immediate reactions and to delayed reactions The delayed reactions are particularly important in such cases It was observed, however, that persons recently vaccinated or tested with vaccine fractions gave stronger reactions than persons not recently vaccinated or tested

All these tests of allergy, therefore, failed to show increased cutaneous reactivity Differentiation between a toxic and an allergic mechanism thus was not possible on the basis of test results Nor could it be made by serologic or histologic studies

II *The individual*

Eruptions resembling erythema multiforme and flare-up of eczema occurred after primary vaccination and after revaccination Complications following revaccination occurred in cases with "normal" local reaction as well as in cases with relatively severe local reaction, and even in two cases in which vaccination did not take The state of individual immunity to vaccinia virus thus did not seem to play a major part in the occurrence of these dermatoses This was also reflected in serologic studies

The interval between vaccination and appearance of complications ranged from one day to four weeks, with an average period of eleven days This is in conformity with reports in the literature

The role of *atopy* can not be clarified by our study It seems, however, to be no correlation between atopy and occurrence of vaccinal complications judging from our study of vaccinated clear cases of atopic dermatitis, allergic rhinitis and allergic asthma

The site of the changes in the skin seems to be important, however Some unknown factor clearly determines where the tissue reaction in the skin shall take

place — in the superficial or deeper vascular regions of the corium It is noteworthy that many of our patients had disturbances of the peripheral circulation, although these could be apparently trivial, for instance, coldness of the extremities, livedo annularis etc

In mass vaccination against smallpox it is not feasible to take into consideration all more or less insignificant cutaneous lesions or general disorders, such as focal or generalized infections, but it would seem advisable to devote more study to the conditions underlying complications of the types here described This might permit greater reliability in deciding contra indications to smallpox vaccination

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From the National Bacteriological Laboratory, the Hospital for Infectious Diseases Stockholm and the Municipal Virological Laboratory, Gothenburg, Sweden

Vaccinia Antigens for Skin Testing Preparation of Antigens and Quantitative Studies of Skin Reactions in "Standard Populations"[1]

LARS NOREN, ÅKE ESPMARK, ASTRID FAGRAEUS, STIG E HOLM, JONAS LINDAHL, ERIK LYCKE and JURGEN MARQUARDT

Following the extensive vaccination campaign in Stockholm in 1963 several cases of postvaccinal skin eruptions of suspected allergic nature were encountered (2) This incited the study on the properties of some preparations of vaccinia antigens *in vitro* and the ability of these preparations of eliciting skin allergic reactions when tested in some normal populations In the sequel preparation of three different test antigens is briefly described as well as skin tests in animals and man, carried out with the following purposes

a) To study the relation between the dose of antigen injected and the size of the cutaneous reaction

b) To study the possible use of this relationship for a quantitative estimation of the allergenic capacity of the vaccinal antigen preparations

c) To try to estimate quantitatively the "normal" skin sensitivity in some groups of individuals with defined history of vaccination or smallpox

[1] This study was partly supported by a grant from Statens Medicinska Forskningsråd

Material and methods

Preparation and in vitro properties of test antigens

Preparation I contained soluble antigens from the liquid phase of vaccinia virus infected calf kidney cultures The virus particles were removed almost completely by centrifugation three times at 23,000 g The last supernatant fluid contained only 3 pock forming units per 0 1 ml as tested on the chorioallantoic membrane Preparation I tested by the diffusion in gel technique against a vaccinia hyperimmune rabbit serum was shown to contain 4 separate precipitinogenic factors

Control antigen consisted of the medium from non infected calf kidney cultures, treated as Preparation I

Preparation II contained concentrated soluble antigens from vaccinia virus infected cultures of embryonic calf skin and muscle tissues (13) The material was centrifuged and prepared by precipitation at pH 4 5 principally according to the technique described by Shedlovsky and Smadel (12) When tested by the diffusion in gel technique

against a vaccinia hyperimmune rabbit serum 7 separate (vaccinial) precipitation bands were recorded The preparation contained no infectious virus

The purified and inactivated virus preparation (Preparation III) was produced from rabbit skin adapted vaccinia virus (6) The initial crude suspension was purified by differential centrifugation and treatment with fluorocarbon Virus was separated in a continuous sucrose gradient from 20 to 50 per cent and was obtained as a distinct band, which was harvested, washed and inactivated by dialysis in 25—50—100 per cent alcohol acetic acid mixture and finally subjected to ultrasonic treatment Electron photo micrograph of unwashed and unshadowed material revealed only minor impurities This preparation contained no infectious virus and was freed from soluble antigens

Complement fixation titers of the above mentioned three antigen preparations tested against 4 antibody units of a known antivaccinia serum are presented in Table 41

Performance of skin tests and quantitative estimation of skin reactions

For skin testing 0 1 ml of the antigen preparations was injected intracutaneously The erythema was recorded after 1—4 and after 24 and 48 hours and its diameter was measured with a calibrated ruler When the erythema was not circular the "average diameter was calculated as the square root of the product of the short and the long axis) Induration was also noted and recorded as the increase in thickness of a skinfold at the site of injection as compared to the thickness of an adjacent skinfold The development of induration was essentially similar to the development of erythema

TABLE 41 Antigenic titers (in CF test against vaccinia positive serum) of test preparations used for skin testing

Antigen preparation	Complement fixation titer
Preparation I	1/4
Preparation II	1/20
Purified virus (Prep III)	Neg
Control antigen	Neg

Intracutaneous tests were performed in unvaccinated and vaccinated guinea pigs in humans without preceding vaccination after uncomplicated vaccination, and in patients recovered from smallpox

Results

Skin tests in guinea pigs

Fifty guinea pigs were immunized with smallpox vaccine prepared from vaccinia virus infected chorioallantoic membranes Another group of 10 animals were not vaccinated but inoculated intracutaneously with extract from uninfected chorioallantoic membranes The vaccinated as well as the unvaccinated guinea pigs were skin tested, using the test antigens described above (Preparation I was injected in dilution 1/2 1/20 and 1/200 Preparation II in dilution 1/10 1/100 and 1/1 000 the purified virus preparation in dilution 1/60 and the control antigen in dilution 1/2) Half

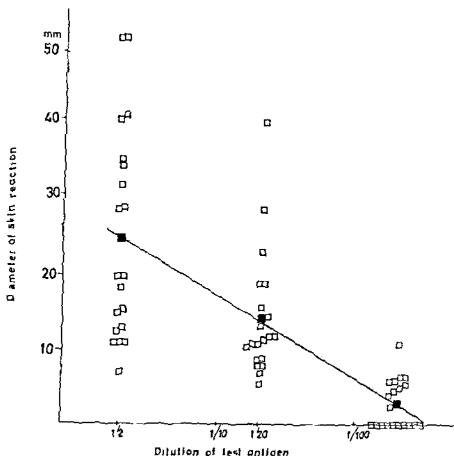


Fig 60 Relationship between dilutions (logarithmic scale) of soluble vaccinia antigen (Prep I) and diameter of skin reactions 48 hours after intracutaneous injection in adult males (recruits) successfully vaccinated 1 month prior to skin testing
☐ Individual values ■ Average within dilutions Regression line fitted to mean values

of the number of animals were tested 5 days after vaccination, the others 14 days after vaccination

In the group of vaccinated guinea pigs a positive delayed skin reaction was found when Preparation II and Preparation I (the soluble antigens) were used The diameter of the skin reaction (erythema) seemed to be directly proportional to the logarithm of the antigen dilution injected

In the unvaccinated control group of 10 animals only one developed a weak delayed reaction to Preparation I diluted 1 2 (12 × 12 mm after 48 hours) and another animal a very weak reaction to Preparation II diluted 1 10 (3 × 3 mm after 48 hours) The other animals of the control group were negative to all the four test antigens

Antibody response to vaccination was measured by the haemagglutination inhibition test (HI test) at the time of skin testing HI antibodies were demonstrated in the majority of the group tested 5 days after vaccination and in all vaccinated animals tested after 14 days No correlation was found between the HI titers and the size of cutaneous reactions

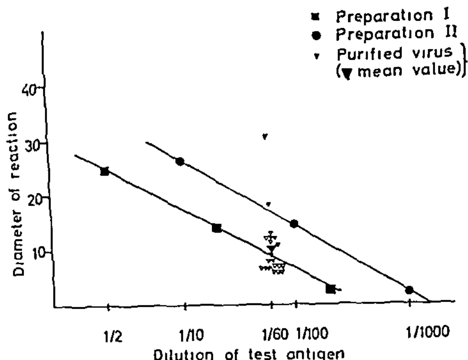


Fig 61 Responses to skin testing in recently vaccinated individuals with different dilutions of soluble vaccinia antigen (mean value) and with one single dilution of purified virus (individual values open triangles)

Skin tests in humans

In a small group of 5 unvaccinated individuals (children aged 5—12 years) none displayed any delayed reactions to the test antigens

Twenty healthy recruits were skin tested one month after successful revaccination Each individual was injected with each of three consecutive tenfold dilutions of Preparations I and II with purified virus diluted 1 60 and with *control antigen diluted 1 2 The purified* virus preparation was calculated to contain about 10^{8} virus particles per *injected dose*

All injected individuals exhibited delayed reactions to Preparations I and II and weaker reactions to the purified virus material One individual in this group developed a weak delayed reaction to the control antigen the others were completely negative for this preparation

Figure 60 shows the size of individual reactions (diameters of erythema after 48 hours) to the three tested dilutions of Preparation I Open squares represent the individual values filled squares the mean values which form a straight line *against the logarithms of the antigen* dilutions The mean values of the skin reactions obtained with preparations I and II are plotted in the regression diagram of Figure 61 together with individual values obtained with the purified virus preparation The figure indicates

TABLE 42 Mean skin titers (dilution estimated to give a 10 mm *reaction*) obtained with different vaccinia antigens in various groups of individuals differing with respect to history of vaccination or smallpox

	Prep I	*Prep II*	Purified virus	Control antigen
Unvaccinated (5 children)	Neg	Neg	Neg	Neg
Twenty recruits (successfully revaccinated 1 month earlier)	1/50	1/220	1/60	Neg
Often vaccinated group (six lab staff members) 3 months after the last vaccination	1/80	1/140	1/30	Neg
Often vaccinated group (four lab staff members) 6 months after the last vaccination	1/10	1/40	1/30	Not done
Eight smallpox patients 5 months after recovery	1/10	1/20	1/30	Neg

that Preparation II contained about 5 times as much "allergen" as Preparation I and the purified virus preparation The latter contained, however, a much larger amount of elementary bodies than Preparation I, and therefore it is reasonable to assume that the elementary bodies played a less active role as an "allergen" Accordingly most of the allergen seemed to be present as soluble antigens

The straight course of the lines in Figure 61 permits an estimation of the "skin titer" of the different antigens "Skin titers" were arbitrarily defined as the dilution of antigen preparation corresponding to a skin reaction with a diameter of 10 mm They will be used below to estimate the relative sensitivity of different populations Some of the "skin titers" were obtained by extrapolation

Antibody response to the preceding vaccination could be demonstrated in all the skin tested individuals, but there was no correlation between the antibody titers in serum (HI and CF titers) and the size of the cutaneous reactions

Eight patients, recovered from variola 5 months prior to this study were skin tested in the same way as the groups described above The cutaneous reactions in this group were weaker for Preparations I and II and slightly weaker for the purified virus preparation than the reactions recorded among the recruits The relatively weak reactions in the variola group was unexpected This group was, however, tested 5 months after recovery from smallpox, i e relatively later than the recruit group Therefore it was considered that the difference might be due to time This assumption was corroborated by comparison of repeated skin tests 3 and 6 months after vaccination in an often vaccinated group of individuals, suggesting that "skin titers" decrease signif

icantly in the course of a few months The "skin titers" obtained in the different tested groups are summarized in Table 42

Discussion

Delayed skin reactions to vaccinia antigen preparations were shown to be significantly dose dependent. A linear relation was obtained between the diameter of the erythema and the logarithm of the injected dose of antigen The results from the skin tests in the guinea pigs were essentially similar to those in human beings, although the reactions in the animals were weaker This is not unexpected, as the delayed type of hypersensitivity in general is known to be particularly pronounced in man (4)

In humans a tenfold increase of the dose corresponded to about 10 mm increase of the diameter of the reaction

However the variation in individual sensitivity within a seemingly homogenous group of individuals was very pronounced In the example of Figure 60 the mean diameters with the two highest doses were 25 and 14 5 mm and the standard deviation of individual values was of the magnitude 14 and 9 mm respectively Hence the dose dependence of reactions may be revealed only if relatively large groups are tested

Obviously the large individual variation will cause similar difficulties when comparing the sensitivity of different categories of individuals For instance a difference between the mean diameter of skin reactions in two groups of 20 persons each has to exceed 7 5 mm in order to be revealed with 95 % confidence with regard to the variation mentioned above

Evidently the use of only ungraded response (+ or —) would be an even less favourable method for detecting differences between groups

In the comparison between the test antigens it was found that the preparations of soluble antigens were more active as allergens than that of purified virus particles This may be partly due to differences in diffusibility and partly to differences in antigenic composition Vaccinia virus materials have been found to contain several immunoprecipitating antigens (11 7) Among the soluble antigen preparations I and II which both contained several antigenic components, the latter was on the average 5 times more potent as an allergen than the former

The difference in antigenicity between the preparations was also reflected in the diffusion in gel tests as only 4 precipitinogens were detected in Preparation I while 7 were demonstrable in Preparation II

However a difference in antigenic potency as demonstrable e g in precipitation analyses must not necessarily correspond to a difference in the ability to provoke allergic reactions Preparation III *contained no soluble antigens* functioning as precipitinogens but could nevertheless provoke skin reactions Yet unpublished results have shown that most if not all the precipitinogens among the soluble antigens correspond to precipitinogenic factors in the virus particles (8)

It seems reasonable to assume that the intact virus particles were not particularly active as allergens The skin reactions observed with Preparation III might be caused by allergens released by degradation of the virus particles intracutaneously

Two of the tested recruits reacted much more strongly to Preparation I and they also had the two largest reactions noted with the purified virus (cf, Figure 61) This may suggest that individuals may differ in their hypersensitivity to different antigenic components This question should be further elucidated by testing purified separate antigen components

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The elements of the positive skin reaction are probably similar in nature to the induration and erythema seen after revaccination and in the later course of the primary reaction (10, 1) From the above dose-response relationship it may be assumed that a large local reaction around a vaccination pock *on the average* signifies a large amount of antigen produced, although this would be difficult to prove by direct means in practise

Similar dose-response relationship have been demonstrated also with tuberculin (5) and the same regression technique has been used for the assay of diphtheria toxin (9) and even live vaccinia virus (3)

The relation between skin sensitivity as determined by intracutaneous testing and the tendency of developing generalized postvaccinal rashes still remains to be investigated

Summary

Cutaneous reactions were studied after injection of three vaccinia antigen preparations in unvaccinated and vaccinated guinea pigs, in humans without preceding vaccination, after uncomplicated vaccination, and in patients recovered from smallpox The elementary body preparation proved to be less active as an "allergen" than the soluble antigen preparations A linear relation was found between the diameter of the reaction and the log-dose of the injected antigens The cutaneous reactions to the soluble antigen preparations decreased significantly during a 3 month period The results revealed no difference in reaction between normally vaccinated persons and patients recovered from smallpox

On the basis of the estimated magnitude of variation among the skin reactions some considerations are presented for evaluation of the possibility of verifying sensitivity differences of various populations

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On the basis of the estimated magni tude of variation among the skin reactions some considerations are presented for evaluation of the possibility of verifying sensitivity differences of various populations

symptoms were sometimes rather disabling, even seven months after the vaccination he still suffered from occasional attacks of asthma However, steroid treatment relieved him of his symptoms

The blood eosinophilia and the transient infiltrations of both lungs correspond with the main criteria of Loffler s syndrome (2) According to Loffler s original definition the disease causes few or no symptoms, lasts only a short time and has a favourable outlook Lately however, several cases have been reported in which the symptoms were severe and the course protracted (1)

Most authors assume that Loffler s syndrome is caused by a reaction between antigen and antibody in the lung tissue Sometimes no definite allergen can be demonstrated but signs of infection with bacteria or parasites may be present Allergy to different kinds of drugs has also been described

In the present case the symptoms were probably caused by an allergic reaction to the smallpox vaccination

Summary

A 37 year old man got an allergic syndrome (transient pulmonary infiltrates and blood eosinophilia) two weeks after vaccination against smallpox The symptoms were rather severe Steroid treatment, however, gave subjective relief

It is assumed that the symptoms were caused by an allergic reaction to the vaccination

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From the Department of Medicine IV (Head Sixten Kallner†, M D) Sodersjukhuset Stockholm, Sweden

A Case of Asthma after Vaccination against Smallpox

KARL EKBOM

A great deal of attention has been given recently to the occasional side effects of vaccination against smallpox

It is the purpose of this paper to describe a patient who got an unusual allergic syndrome after being vaccinated against smallpox There seem to be no previous reports of similar cases

Case report

A 37-year-old man was admitted on Dec 10, 1963 with a history of dyspnea and distressing attacks of asthma

He had been well until his present illness and the family history revealed no allergic diseases

He was vaccinated against smallpox when he was a child and was revaccinated at the age of 20 During the smallpox epidemic in Stockholm he was vaccinated once more in May, 1963 He was quite healthy at that time and showed no signs of infection The vaccination became positive, but about two weeks after the vaccination he began to suffer from attacks of asthma, a dry cough and shortness of breath on physical exertion He was unable to do any work Symptomatic treatment gave little relief

Roentgen examination of the lungs on Sept 27 showed nothing abnormal

Physical examination on admission revealed occasional sibilant rales over the lung fields but a good general condition and nothing else of note

Laboratory data hemoglobin 14 4 Gm per cent, W B C 6,700 per mm³, total eosinophils considerably raised, 1,517 per mm³, the sputum contained many eosinophils but no pathogenic bacteria or tubercle bacilli The Mantoux reaction was negative to 0 01 mg Guinea pig inoculations and cultures of gastric irrigation fluid were negative for tubercle bacilli

The ECG was normal Another roentgenographic examination of the lungs on Dec 12 revealed confluent densities in the parenchyma above the hilus on the left side, and lateral to the hilus on the right side On re examination a week later the infiltrates on the right side had vanished and the ones on the left had grown much smaller After another two weeks the lesions had disappeared entirely

He was then given 8 mg of Triamcinolone (Kenacort®) daily and after this the asthma vanished On re-examination four months later he was still free of symptoms with this dosage

Discussion

In this case, an allergic syndrome developed after the vaccination The patient had been quite healthy previously and had shown no signs of allergy His



ACTA MEDICA SCANDINAVICA

SUPPLEMENTUM 465

PRE-EXCITATION

STUDIES ON CRITERIA, PROGNOSIS AND HEREDITY

by

ERIK ORINIUS

STOCKHOLM 1966

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Tryckeri Balder AB, Stockholm 1966

CONTENTS

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INTRODUCTION

By pre excitation is meant a premature activation of a portion of the ventricular myocardium following atrial systole This anomaly manifests itself by electrocardiographic changes (a short P R interval initial slurring of the QRS complex called a delta wave a wide QRS complex S-T depression and T wave inversion) The anomaly does not by itself give rise to any symptoms but it is often associated with paroxysmal tachycardia The electrocardiographic changes in combination with the proneness for attacks of tachycardia were described for the first time by Wolff Parkinson and White in 1930 (57) The term pre excitation was introduced by Öhnell in 1944 (34)

The pathophysiology of pre-excitation is still obscure as is the morbid anatomy Many theories have been brought forward and they may be divided into three groups (5) a) the atrial impulse reaches the ventricles first through an accessory atrioventricular (A V) communication and soon thereafter through the normal pathway b) the atrial impulse is less delayed in one part of the A V node than in the remainder thereby giving rise to two ventricular excitation waves c) atrial systole activates a septal focus electrically or mechanically and this impulse forms a fusion complex with that transmitted by the Purkinje fibers At present the by pass theory seems to be most generally accepted (15 37 59) and various accessory A V connections have been demonstrable in some cases at autopsy (27)

The genetic basis of pre excitation is still obscure In at least one family evidence has been presented for pre excitation being an inherited anomaly (20) but in another study on several families with pre excitation members no additional cases were found (54)

The frequency of pre excitation in the general population is not known but according to studies from recent years it occurs in about 1 5 ‰ of the cases in electrocardiogram archives (22 25) Approximately 60 % of patients with pre-excitation electrocardiograms suffer from paroxysmal supraventricular tachycardia (15 22 25 34 56 58)

Pre excitation is important to the physician for two reasons it may explain a paroxysmal tachycardia as part of the pre excitation syndrome and it may simulate or mask the electrocardiographic pattern of other cardiac disorders (ectopic atrial rhythm intraventricular conduction disturbances coronary insufficiency myocardial infarction) (12 34 36 49 59) The diagnosis is based on a short P R interval a delta wave and a wide QRS complex (34 57) Through the years there has been a tendency however to waive either the requirement for a short P R interval or for a broad QRS complex (cf 57 34 15) No systematic studies of the configurational criteria seem to have been published

The prognosis in pre excitation is usually said to be good when not associated with frequent or prolonged attacks of tachycardia

As electrocardiographic criteria for their syndrome Wolff Parkinson and White (57) required that the P R interval should be 10 csec[1] at the most and that the QRS complexes should be aberrant as in complete or incomplete bundle branch block. Later Öhnell (34) developed the electrocardiographic diagnostics of pre excitation and proposed the following configurational criteria a P R interval of $\leq$ 12 csec + a *delta wave in at least two standard leads with a duration of* $\geq$ 5 csec and a slope of 3—8 mV/sec + a QRS complex with a duration of > 10 csec and an amplitude of > 0 8 mV in at least one lead Even in some subjects with a P R interval of $\geq$ 13 csec or a QRS-duration of $\leq$ 10 csec Öhnell (34) considered pre excitation to be proven by the appearance of one of two electrocardiographic phenomena which he had observed in cases fulfilling the configurational criteria the concertina phenomenon i e the increasing or decreasing of the delta wave duration from one QRS complex to another and the genuine normalization phenomenon i e the complete disappearance of the delta wave from one QRS complex to another By using these two pre excitation characteristics the diagnosis has been made in cases with P R intervals of up to 16 csec (44) Thus the delta wave is the only electrocardiographic change without which the diagnosis of pre excitation has not been made This agrees with what might be expected theoretically a premature excitation of a portion of the ventricular myocardium would cause a shortened P R interval followed by a pre wave The P R interval need not, however be shortened to as little as 12 csec.

These considerations raise the question of whether a delta wave *without a co existing* short P R interval is sufficient for the diagnosis of pre excitation *without the support* of genuine normalization or the concertina phenomenon. Furthermore the question arises as to how short these isolated delta waves may be without losing their significance as regards pre excitation Both these questions could be answered by comparing the frequency of delta waves of different durations in previously diagnosed pre-excitation subjects and in controls on the one hand and in patients with paroxysmal tachycardia and in controls on the other Subjects with previously diagnosed pre excitation are suitable for this purpose as the duration of a delta wave may vary spontaneously and patients with paroxysmal tachycardia as thus forms the other part of the pre excitation syndrome

Besides the P R shortening and the delta wave the anomalous ventricular excitation in pre-excitation may give rise to widening of the QRS complex, S-T depression and T wave inversion As these changes are less specific than the delta wave their possible importance in the diagnosis of pre excitation will not be examined further

[1] 1 csec = 0 01 sec

(5, 15, 22, 23, 59) The background for this reservation is the fact that occasional patients have died during an attack of tachycardia (26, 34, 45) No systematic follow up studies with long observation times seem to be reported

Pre excitation has been reported to co exist with various forms of heart disease, in children especially frequently with Ebstein s malformation (42), and in adults with ob structive cardiomyopathy (10)

Thus, there are many problems unsolved with regard to pre excitation, and *the pur pose of this study* has been

a) to develop the configurational criteria for a pre excitation electrocardiogram,

b) to study the long term prognosis in pre excitation with reference to mortality, pa roxysmal tachycardia and co existing car diac disease, and

c) to study the possible genetic basis of pre excitation

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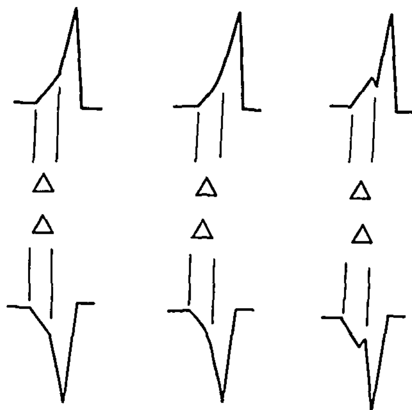


Fig 1 Different configurations of delta waves with the durations indicated

Delta waves in re-examined pre-excitation subjects and in controls

Material

Two groups of pre excitation subjects were available for re examination, Öhnell s from 1944 (34) and Biorck s from 1946 (8), together comprising 75 cases Sixty nine of these could be traced and 45 of them were still alive in 1965 (table 9, page 25) All these subjects were invited to a re examination including the recording of an electrocardiogram to which 38 persons came Three more could be followed up through the assistance of their physicians who kindly sent the latest electrocardiogram Thus the electrocardiograms in 41 cases (91 %) altogether were acquired and examined The patients were between 29 and 84 years old 24 we e males and 17 females (Further details concerning the subjects are to be found on page 24 and in Appendix I)

In 15 of these 41 pre excitation subjects Öhnell (34) and Biorck (8) had based their diagnoses on Wolff Parkinson White s and Öhnell s configurational criteria and in the other 26 cases on spontaneous or provoked genuine normalization and the concertina phenomenon

The matched controls for the pre excita tion subjects not more than 65 years old were taken from a general health survey of civil servants which had been performed at Serafimerlasarettet a hospital in Stockholm in 1965 The controls had to be of the same sex and the difference in age was not to exceed three years The health survey files

Number of	pre excitation subjects	controls
with a delta wave of		
2 csec	2	2
3	1	0
4	4	0
5	1	0

Table 1 The occurrence of delta waves with different durations at re examination of 26 previously diagnosed cases of pre-excitation and among 26 matched controls from a health survey

were serially reviewed according to these criteria until a control had been found for each pre excitation subject Cases with electrocardiographic changes in the P R or delta wave region (atrial fibrillation bundle branch block) were avoided but no cases were excluded for any other abnormality For the five cases more than 65 years old matched controls were selected at random from a private health examination centre (Metropol Stockholm) In one of these subjects the difference in age amounted to 7 years

Methods

The electrocardiographs used at re examination were in almost all cases of the ink writing type (Mingograph Elema Schönander Stockholm) Routinely leads I II III CR_1 2 4 5 7 V_1 2 4 5 and 7 were recorded on 4 channels for about 10 seconds with a paper speed of 50 mm/sec

The control electrocardiograms too had been recorded by a 4 channel ink writing apparatus (Mingograph Elema Schönander Stockholm) Routinely leads I II III CR_1 2 4 5 and 7 and V_1 2 4 5 and 7 were recorded with a paper speed of 50 mm/sec The available electrocardiographic strips included 2—5 complexes in each lead

The electrocardiograms of the pre excitation subjects and the controls were examined as regards delta waves in leads I II III CR_1 2 4 5 7 and V_1 2 4 5 7 The criteria for a delta wave were 1 the upstroke of the R wave or the downstroke of the QS wave should consist of two parts with different slopes the first of which should be less steep (fig 1) 2 the delta wave should occur in two or more leads the CR and V leads being considered to be equal 3 if the delta wave did not occur in all leads it should start before the QRS complexes in the leads without a delta wave (When doing so the fact that the channels are not always synchronous was taken into consideration) The delta waves were measured with regard to their duration and slope in the lead where they were most conspicuous (I II CR/V_2 4 and 5) In the same lead the P R and QRS intervals as well as the ST J depression if any were measured All intervals were measured to the nearest 1 csec and the ST J depressions to the nearest 0 5 mm and the results are given as the average of three complexes Delta waves of 1 csec were ignored being difficult to discern Electrocardiograms with delta waves were also examined for the occurrence of genuine normalization Examina

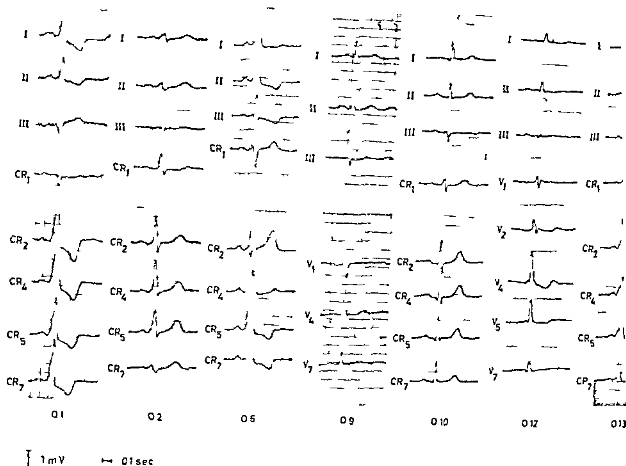


Fig 2 A Electrocardiograms from the follow up examination of 41 pre excitation subjects Ö 1 etc = the case numbers in Öhnell s report (34)

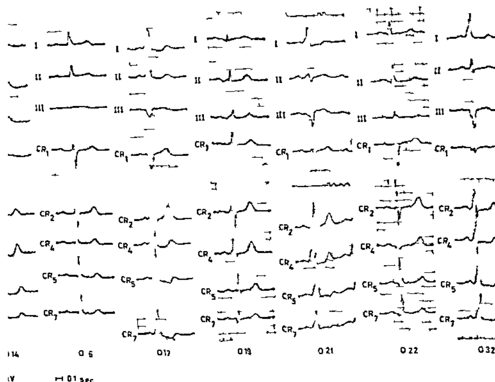
tion for the concertina phenomenon was not done being considered too unreliable with delta waves as short as 3 csec

Results

At the re examination 5 of the 41 pre excitation subjects fulfilled Wolff Parkinson White s criteria (Öhnell s case no 13 32 37 42 and 54 see Appendix I) and 5 others Öhnell s configurational criteria (Ö 14 17 21 41 and 49) Genuine normalization occurred intermittently in two cases (Ö 9 and 49) one of which (Ö 49) also fulfilled the configurational criteria Altogether 11 (27 %) were diagnosed by these criteria

Four more cases had a P R interval of 11—12 csec and a delta wave at re examination (Ö 6 40 50 and Biorck s case no 2) but owing to the delta wave having too short a duration or too steep a slope they did not fulfill Öhnell s configurational criteria

Twenty six previously diagnosed pre excitation subjects with a P R interval of ≥ 13 csec at re examination remained to be compared with as many controls with regard to the frequency of isolated delta



ig. B Electrocardiograms from the follow up examination of 41 pre excitation subjects Ö 14 etc = he case numbers in Öhnell s report (34)

waves Of these 26 17 were males and 9 females aged 29—84 years

As can be seen in table 1 there were 8 cases with isolated delta waves with a duration of > 2 csec in the pre excitation group and two cases among the controls The difference is not significant. As no delta waves exceeded 2 csec in the control group the frequencies of delta waves of > 3 csec also were compared Delta waves of these durations were found in 6 subjects (Ö 1 2 12 19 43 and 44) in the pre excitation group but in none among the controls here the difference is probably significant ($P < 0.05$ χ^2)

The PR interval for the 6 subjects with a delta wave of ≥ 3 csec. was 13—14 csec and the QRS interval 12—15 csec In 4 of these 6 cases the S-T changes could not be judged with reference to pre-excitation as the patients were on digitalis or were in the age group where coronary heart disease is common In one of the remaining cases no S-T depression was recorded and in the other it was 0 5 mm No intermittent genuine normalization (34) was observed

For details see figs 2 A F and Appendix I

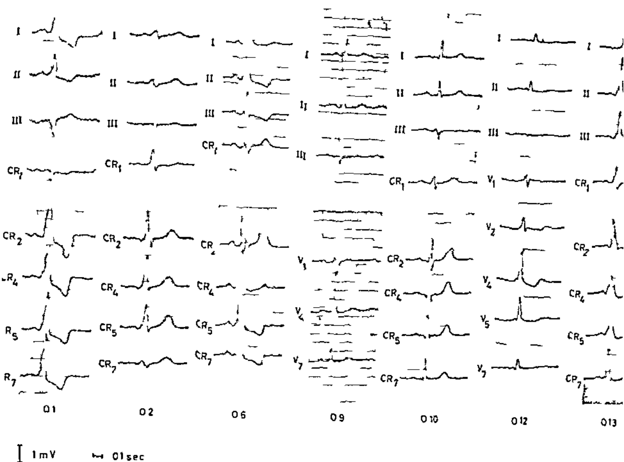


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Ö 54 Ö 55 Ö 56 Ö 58 Ö 60 Ö 62 Ö 65

1 mV 0 1 sec

Fig 2 E Electrocardiograms from the follow up examination of 41 pre excitation subjects Ö 54 etc = the case numbers in Öhnell s report (34)

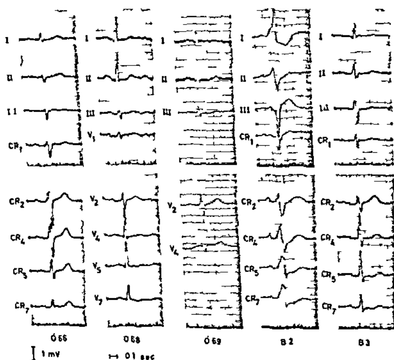


Fig 2 F Electrocardiograms from the follow up examination of 41 pre excitation subjects Ö 66 etc and B etc = the case numbers in Öhnell s and Böröck s reports (34, 8)

I 1 mV ⟼ 01 sec

Fig 2 C Electrocardiograms from the follow up examination of 41 pre excitation subjects Ö 35 etc = the case numbers in Öhnell s report (34)

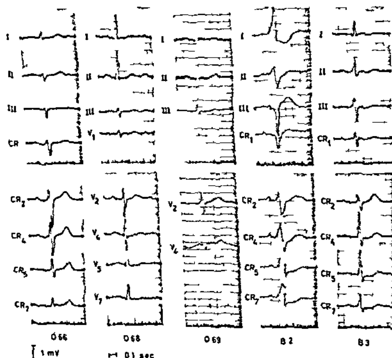
I 1 mV ⟼ 01 sec

Fig 2 D Electrocardiograms from the follow up examination of 41 pre-excitation subjects Ö 42 etc = the case numbers in Öhnell s report (34)

Ö 54 Ö 55 Ö 56 Ö 58 Ö 60 Ö 62 Ö 65

mV 0 1 sec

F g 2 E Electrocard ograms f om the follow up exam nation of 41 pre-excitation sub ects Ö 54 etc. = he case numb s in Öhnell s report (34)



F g F Electrocard grams f om the follow up examinat on of 41 pre-excitation sub ects Ö 66 etc and B 2 et = the case numbe s n Öhnell s and Biorck s reports (34 8)

Number of pre excitation subjects	with digitalis	without digitalis	P	with quinidine	without quinidine	P
with a delta wave of at least 3 csec	2	4		0	6	
without a delta wave of at least 3 csec	4	16	>0 1	1	19	>0 1

Table 2 The occurrence of delta waves of ≥ 3 csec and digitalis/quinidine treatment at re examination of 26 previously diagnosed cases of pre excitation P values from χ^2-tests

Comments

Judging from the reproduced electrocardiograms (8, 34) the original diagnosis of pre excitation seems to be well founded in every case Still one could expect some cases not to show a pre excitation electrocardiogram at re examination as pre excitation is a changing condition (genuine normalization and concertina phenomenon) (34) However, the proportion of cases without a pre excitation electrocardiogram at re examination, 73 %, seems to be remarkably large

No case of intermittent genuine normalization was recorded among the 6 cases with isolated delta waves ≥ 3 csec and this frequency does not diverge from one case out of the 10 that fulfilled Wolff Parkinson White s or Öhnell s criteria

Among the pre excitation cases delta waves of ≥ 3 csec were thus probably over represented As this over representation might be caused by treatment with cardiac drugs the medication was compared for the subjects with and without a delta wave of ≥ 3 csec

As seen in table 2 the subjects with and without a delta wave do not differ significantly with reference to cardiac medication, and the over representation of delta waves cannot be explained as a drug effect

Delta waves in patients with paroxysmal tachycardia and in controls

Material

All the available records from 1958 to 1965 with the diagnosis of paroxysmal supraventricular tachycardia from the departments of medicine at Serafimerlasarettet and Karolinska sjukhuset were studied The patients accepted for this study were those who had a history of two or more attacks of very rapid palpitations of sudden onset and had been examined during an attack, at which time an electrocardiogram showed a regular supraventricular tachycardia or auscultation/palpation revealed a regular tachycardia, the lowest rate being 130/min in both cases Among the electrocardiograms in these records the first without tachycardia was selected for examination Three cases with a P R interval of ≤ 12 csec were excluded as this study is concerned with delta waves without co existing short P R intervals Thirty one patients remained 16 males and 15 females varying in age from

17 to 75 years For 25 of these there existed electrocardiograms which had been taken during an attack of tachycardia and showed these to be of supraventricular origin with regular rates of 130—220/min In the 6 remaining patients the heart rate had been auscultated or palpated and judged to be regular with a rate of 130—220/min.

Controls for the patients with paroxysmal tachycardia, who were less than 65 years old were obtained from a general health survey of civil servants performed at Serafimer lasarettet in 1965 The same sex and a difference of age not exceeding three years were the requirements for the controls The health survey files were serially reviewed according to these criteria until each patient with paroxysmal tachycardia had been provided with a control For the three patients aged over 65 controls of the same sex were selected at random from a private health examination centre (Metropol Stockholm) In one of these cases the difference of age amounted to 7 years

Methods

All electrocardiograms were recorded by a 4 channel ink writing apparatus (Mingograph Elema Schonander Stockholm) Routinely leads I II III $CR_{1\ 2\ 4\ 5\ 7}$ $V_{1\ 2\ 4\ 5}$ and $_7$ had been recorded with a paper speed of 50 mm/sec The available electrocardiographic strips included 2—6 complexes in each lead

The electrocardiograms from the tachycardia patients and the controls were scrutinized with reference to delta waves in leads I II III $CR_{1\ 2\ 4\ 5\ 7}$ and $V_{1\ 2\ 4\ 5\ 7}$ The criteria for a delta wave were the same as those used for the pre excitation group the upstroke of the R wave or the downstroke of the QS wave should consist of two parts with different slope the first of which should be less steep (fig 1), the delta wave should occur in two or more leads the CR and V leads being considered equal if the delta wave did not occur in all leads it should start before the QRS complexes in the leads without a delta wave (When doing so the fact that the channels are not always synchronous was taken into consideration) The duration and the slope of the delta wave were measured in the lead where they were most conspicuous (II,CR_5) In the same lead the P R and QRS intervals and occuring S-T depressions were measured All intervals were measured to the nearest 1 csec and the ST J depressions to the nearest 0 5 mm and the results are given as the average of two complexes Delta waves of 1 csec were ignored being difficult to discern The electrocardiograms with delta waves were also examined as to the occurrence of genuine normalization Examination for the concertina phenomenon was not done being considered too unreliable with delta waves as short as 3 csec

Results

As can be seen in table 3 7 cases with isolated delta waves of $\geq$ 2 csec. occurred in the patients with paroxysmal tachycardia and three among the controls This difference is not significant Six cases with a duration of $\geq$ 3 csec were found among the patients with paroxysmal tachycardia but none in the controls here the difference is probably significant ($P < 0.05$ χ^2)

The P R intervals of the 6 patients with a delta wave of $\geq$ 3 csec. were 13—17 csec The QRS interval varied between 8 and 11 csec The S-T changes could not be judged with reference to pre excitation in any case as the patients either were on digitalis or

Number of	patients with paroxysmal tachycardia	controls
with a delta wave of		
2 csec	1	3
3	3	0
4	2	0
5	1	0

Table 3 The occurrence of delta waves with different durations among 31 patients with paroxysmal *tachycardia and 31 matched controls from a health survey*

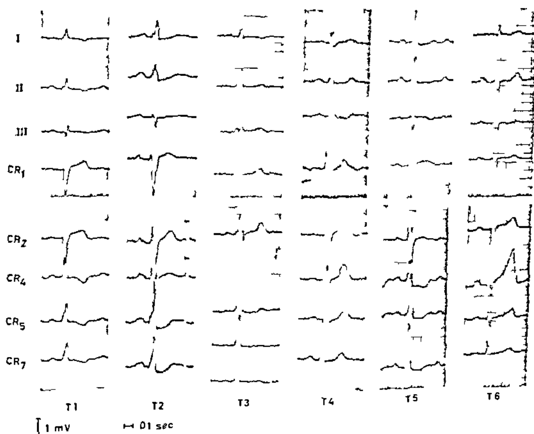


Fig 2 Electrocardiograms from the 6 paroxysmal tachycardia patients with delta waves and normal P R intervals T 1 etc refers to appendix II

were in an age group where coronary heart disease is common

Intermittent genuine normalization (34) was *not observed* For details see fig 3 and Appendix II

Comments

As the electrocardiograms from the hospital records *only* included 2—6 complexes in each lead the chances that intermittent genuine *normalization* could be

Number of patients with paroxysmal tachycardia	with digitalis	without digitalis	P	with quinidine	without quinidine	P
with a delta wave of at least 3 csec	4	2		0	6	
			>0 1			>0 1
without a delta wave of at least 3 csec	16	9		4	21	

Table 4 The occurrence of delta waves of ≥ 3 csec. and digitalis/quinidine treatment among 31 pa tients with paroxysmal tachycardia and 31 matched control from a health survey P values from χ-tests

observed were very small However no case of genuine normalization among these 6 patients with isolated delta waves does not differ significantly from one among 10 cases of pre excitation (p 10) ($P > 0\,1$ χ^2)

As the over representation of delta waves of ≥ 3 csec among the patients with pa roxysmal tachycardia might be due to car diac medication the occurrence of such treatment has been studied

As seen in table 4 the cases with a delta wave do not differ significantly from those without as far as cardiac medication is con cerned and the over representation of delta waves among the patients with paroxysmal tachycardia can therefore not be explained as a drug effect

Discussion of the methods

The configurational criterion for a delta wave used in this study (p 9 and fig 1) was fulfilled by 95 per cent of the 75 pa tients in Ohnell s and Biörck s reports ac cording to the reproduced electrocardiograms (31 8) The requirement that the delta wave should occur in at least two of the studied leads I II III CR or V_1 2 4 5 and 7 was justified by the results obtained in the 41 re examined pre excitation subjects (p 10) and in 1000 consecutive subjects from the previously mentioned health survey

As seen in table 5 the requirement of a del a wave in two leads would compared with a delta wave in three leads render a diagnosis in 5 more pre excitation cases but in no additional case among the health sur vey subjects On the other hand the use of a delta wave in one lead as compared with in two leads adds only one extra case from the pre excitation group but 4 more f om the health survey cases Thus the optimal minimum number of leads with delta waves was considered to be two

The characteristics of the electrocardio-graphs used (Mingograph Elema Schö nander Sockholm) were good (linearity ± 10 mm and flat frequency response up to 500 c/s) and the paper speed used was high (50 mm/sec)

The random error in measuring the dura tion of the delta waves was determined from two separate examinations of 10 randomly selected electrocardiograms with delta waves

Number of	patients with paroxysmal tachycardia	controls
with a delta wave of		
2 csec	1	3
3	3	0
4	2	0
5	1	0

Table 3 The occurrence of delta waves with different durations among 31 patients with paroxysmal tachycardia and 31 matched controls from a health survey

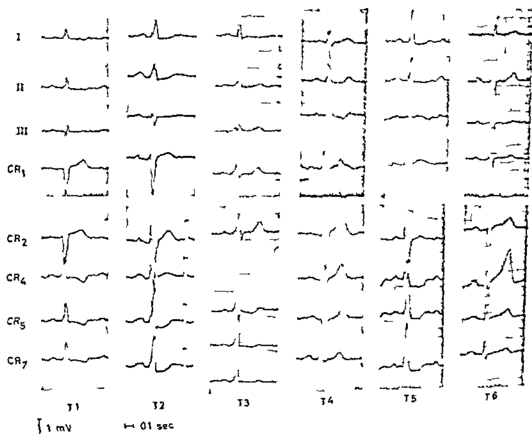


Fig 3 Electrocardiograms from the 6 paroxysmal tachycardia patients with delta waves and normal *PR* intervals T 1 etc refers to appendix II

were in an age group where coronary heart disease is common

Intermittent genuine normalization (34) was not observed For details, see fig 3 and Appendix II

Comments

As the electrocardiograms from the hospital records only included 2—6 complexes in each lead the chances that intermittent genuine normalization could be

Per cent of electrocardiograms with a delta wave in lead	I	II	III	CR_1	CR_2	CR_4	CR_5	CR_7
among W P W/pre excitation cases (n=10 or 9*)	40	30	40	22*	56*	80	89*	60
among cases with an isolated delta wave (n=16)	25	50	31	19	50	63	44	44

Table 6 The occurrence of delta waves in different leads among 10 pre excitation cases fulfilling Wolff Parkinson White s or Öhnell s criteria and among 16 cases with P R intervals of ≥ 13 csec and delta waves of ≥ 3 csec

waves Co existing genuine normalization (34) would also have implied pre excitation as the cause of these delta waves but has not been observed If delta waves in cases fulfilling the conventional criteria were found to be distributed in the recorded leads in another way than in cases with the delta waves discussed this would form evidence against pre excitation being their cause In table 6 the distribution of the delta waves in the different leads is presented for the 10 pre excitation cases according to Wolff Parkinson White s or Öhnell s criteria (p 10) and for the 16 cases with an isolated delta wave in the pre excitation and tachycardia groups (p 11 and 15) The difference in distribution of the delta waves between these two groups is not significant ($P > 0.05$ χ summation)

Comparison of the conventional and the discussed criteria

The risk of accepting a delta wave occurring in at least two leads and with a duration of 3 csec or more in at least one of them, as the electrocardiographic criteria for pre excitation is false positive diagnoses This risk was estimated by studying the frequency of such delta waves in electrocardiograms from two health surveys and from a hospital archives

Health survey electrocardiograms From a general health survey of civil servants at Serafimerlasarettet in 1965 1300 electrocardiograms were obtained and from a general health survey in Stockholm in 1962 (19) another 1000 Most of the participants of these health surveys were not selected at random from their populations The electrocardiograms were recorded with a 4 channel ink writing apparatus (Mingograph Elema Schonander Stockholm) with a paper speed of 50 mm/sec They were examined with reference to delta waves according to the criteria on p 9 in leads I II III CR_1 2 4 5 and 7 Among these 2300 electrocardiograms two pre excitation cases were found which fulfilled Wolff Parkinson White s or Öhnell s configurational criteria and two more which fulfilled the criteria discussed above There are no reports in the literature about the frequency of pre excitation in the general po

Number of	pre excitation subjects	health survey subjects
with a *delta wave* in at least *one lead* with a duration of 3 csec or more	22	6
with a *delta wave* in at least *two leads*, its duration being 3 csec or more in at least one of them	21	2
with a *delta wave* in at least *three leads* its duration being 3 csec or more in at least one of them	16	2

Table 5 The occurrence of delta waves of ≥ 3 csec in ≥ 1 ≥ 2 and ≥ 3 leads at re-examination of 41 previously diagnosed cases of pre excitation and among *1000 cases from a health survey*

and was found to be 0 3 csec No systematic error in measuring the delta wave could be established, a comparison of the mean of my first determination in these 10 electrocardiograms, 4 6 ± 0 34 csec did not show any significant differences from the means found by three trained interpreters 4 5 ± 0 34 4 9 ± 0 28 and 4 6 ± 0 31 csec

Discussion of the results

There was a probable over representation of isolated delta waves of 3 csec or more among the cases with previously diagnosed pre excitation as well as among the cases with paroxysmal tachycardia as compared with the controls (p 11 and 15) The subjectivity of comparative studies of electrocardiograms had been reduced by fixed criteria for the configuration and occurrence of the delta waves No systematic error in measuring the duration of the delta waves could be established and the random error was small Originally the pre excitation material probably included most of the cases with pre excitation from a number of Swedish hospitals and it may therefore be representative of hospital cases with this anomaly For the same reason the patient group with paroxysmal tachycardia may be considered as representative of hospital cases with this disease Matched controls were selected at random from a general health survey of civil servants Civil servants cannot be presumed to differ from the general population with regard to pre excitation Thus the representativity of the subject groups and the precision of the methods seem to allow for the conclusion that an isolated delta wave which occurs in at least two leads and has a duration of 3 csec or more in at least one of them probably is an abnormal finding associated with pre excitation and paroxysmal tachycardia

It seems impossible to decide whether these delta waves always are caused by pre excitation or if they sometimes are a manifestation of altered ventricular activation caused by myocardial infarction or bundle branch block A co existing concertina phenomenon (34) would have presented strong support for pre excitation being the cause of these delta waves but this phenomenon was regarded as too difficult to study in connection with so short delta

Per cent of electrocardiograms with a delta wave in lead	I	II	III	CR_1	CR_2	CR_4	CR_5	CR_7
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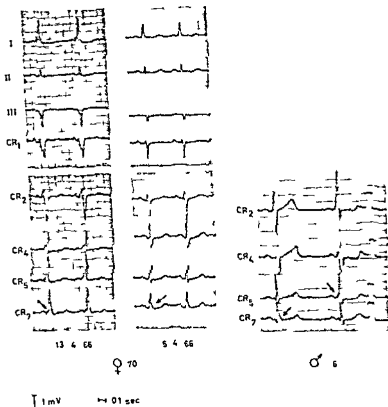


Fig 4 Electrocardiograms from two patients with a delta wave alternating with a terminal delta wave

With these criteria 19 pre excitation cases were found as seen above among 5000 in or out patient electrocardiograms (3 8 ⁰/₀₀) from a hospital which has one department of medicine and one of surgery In these the P R interval was 9—17 csec the duration of the delta wave 3—7 csec and the QRS interval 8—15 csec As the patients either belonged to an age group where coronary heart disease frequently occurs or were on digitalis it was not possible to judge S-T changes with reference to pre excitation in more than 6 cases of which two showed no ST J depression whereas it was 1 mm in two cases and 2 mm in two Paroxysmal tachycardia occurred in 10 (53 %) Fourteen of the 19 were males (74 %) which is a probable over representation in comparison with the 53 % males in the archives as a whole

Similar studies on pre excitation criteria have not been found in the literature for comparison

Terminal delta waves

Öhnell (33) found a gradually descending terminal portion of the QRS complex in some members of two pre excitation families

pulation with which the results here could be compared (For further details, see Appendix III)

Hospital electrocardiograms From the electrocardiogram archives at Serafimerlasarettet about 5000 in or out patient electrocardiograms were selected at random They had been recorded in the same way as the health survey electrocardiograms and they were examined in the same way (p 19) Wolff Parkinson White s or Öhnell s configurational criteria were fulfilled by 10 patients and the criteria discussed above by another 9 Ten pre excitation cases fulfilling Wolff-Parkinson White s or Öhnell s configurational criteria are equivalent to a frequency of 2 $^0/_{00}$ This figure agrees with the frequency of pre excitation reported from other hospitals in recent years (22, 25) *Paroxysmal* tachycardia was found to occur in 7 of the 10 who were diagnosed by Wolff Parkinson White s or Öhnell s *configurational criteria* (70 %) and in three of the 9 who fulfilled only the criteria discussed above (33 %) There is no significant difference between these frequencies ($P > 0.1$, χ^2) (For further details, see Appendix IV)

There is no difference between two cases in the health survey group fulfilling Wolff Parkinson White s criteria plus two fulfilling the criteria discussed and 10 plus 9 in the hospital group, and in the following discussion the hospital figures will be used These figures indicate a risk of up to 9 false positive diagnoses per 10 pre excitation cases fulfilling Wolff Parkinson White s or Öhnell s configurational criteria For a clinical *evaluation of the criteria* discussed, this risk of false positive diagnoses should be compared with the actual frequency of false negative diagnoses with the conventional criteria This frequency was estimated from the re examination of the 41 pre-excitation subjects diagnosed previously Ten of these fulfilled Wolff Parkinson White s or Öhnell s configurational criteria whereas the application of the criteria discussed raised the figure to 21 This means 11 false negative diagnoses with the conventional criteria per 10 pre excitation cases

This under estimation does not substantially exceed the maximum risk of false positive diagnoses which risk therefore will be considered further The detection of pre excitation is of clinical importance mainly in cases of paroxysmal tachycardia and of ST T changes As both of these are over represented in pre excitation, their presence will reduce the risk of false positive diagnoses with the criteria discussed This risk will then probably *be well below the frequency of false* negative diagnoses with the conventional criteria

Not only the risk *but also the* conse quences of false positive diagnoses of pre excitation must be taken into consideration The diagnosis of pre excitation does not exclude other diagnoses such as thyreotoxicosis in patients with paroxysmal tachycardia and coronary insufficiency in patients with ST T changes and it does not in itself call for any action Nor is the prophylaxis and the therapy of paroxysmal tachycardia changed by the diagnosis of pre excitation *The prac*tical consequences of this diagnosis are thus small Yet it is of importance as it will re*duce the groups of* otherwise unexplained paroxysmal tachycardia and ST T changes

In conclusion it seems clinically advantageous to have the following criteria for the diagnosis of pre excitation delta waves in at least two leads with a duration of 3 csec or more in at least one of them (independent of the P R interval)

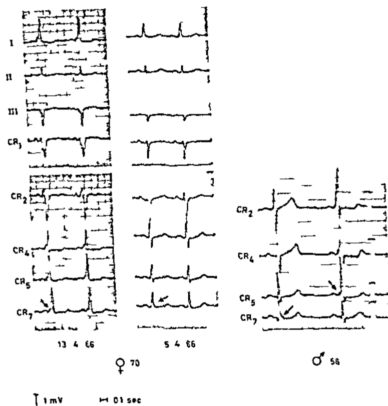


Fig. 4 Electrocardiograms from two patients with a delta wave alternating with a terminal delta wave

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Öhnell (33) found a gradually descending terminal portion of the QRS complex in some members of two pre excitation families

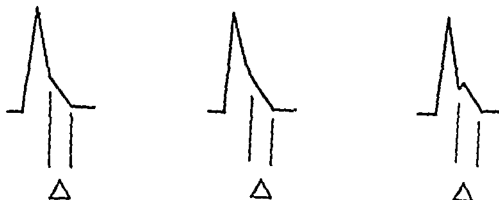


Fig 5 Different configurations of terminal delta waves with the durations indicated

but he did not further investigate whether these terminal delta waves were a manifestation of pre excitation like the initial ones

That there may be a connection between such terminal delta waves and pre excitation is clear from cases such as in fig 4 where a terminal delta wave appears when the initial one disappears Hence it was considered important to study whether terminal delta waves are a manifestation of pre excitation

The aim of this study was to determine whether there is an over representation of terminal delta waves in groups of previously diagnosed pre excitation and paroxysmal tachycardia, each compared with one control group

Material

From the 26 subjects with previously diagnosed pre excitation (p 11) 6 fulfilled my criteria for pre excitation as did 6 from the 31 subjects with paroxysmal tachycardia (p 15) The electrocardiograms of the remaining 20 pre excitation subjects the 25 tachycardia patients and their controls were used for this study

Methods

The leads studied were I, II III CR and $V_{1\ 2\ 4\ 5}$ and $_7$ and the criteria for a terminal delta wave were 1 that the down stroke of the R wave or the upstroke of the S wave should consist of two parts with different slopes the latter of which should be less steep (fig 5), 2 that the delta wave should occur in two or more leads the CR and V leads being considered equal The duration of the terminal delta waves was measured as the average of two complexes in the lead where they were most conspicuous

Results

As can be seen in tables 7 and 8 there was no significant difference between the pre excitation material and the controls either in regard to terminal delta waves of ≥ 2 ≥ 3 ≥ 4 or ≥ 5 csec Nor were there any significant differences between the cases with paroxysmal tachycardia and their controls

Discussion

An alternation from an initial to a terminal delta wave could be explained as a

Number of	pre excitation subjects	controls
with a terminal delta wave of		
2 csec	0	0
3	3	0
4	0	1
5	1	1

Table 7 The occurrence of terminal delta waves with different durations among 20 normalized pre excitation cases and 20 matched controls from a health survey

Number of	patients with paroxysmal tachycardia	controls
with a terminal delta wave of		
2 csec	3	1
3	2	0
4	0	2
5	0	0

Table 8 The occurrence of terminal delta waves with different durations among 25 patients with paroxysmal tachycardia and 25 matched controls from a health survey

pre excitation manifestation if one postulates that the QRS complex in this anomaly consists of a fusion between one Purkinje transmitted and one muscle propagated ventricular activation According to their time relations the two QRS parts could change positions with one another the initial and terminal delta waves representing the extremes

However no over representation of terminal delta waves among the cases with previously diagnosed pre excitation or paroxysmal tachycardia could be shown and a terminal delta wave does therefore not seem to be a manifestation of pre excitation No studies on terminal delta waves have been found in the literature for comparison

The alternation between an initial and a terminal delta wave could also be explained in another way The terminal QRS portion originates from the basal ventricular portion (17) This can also be the pre excited portion (21) and when pre excited it cannot as otherwise be activated terminally

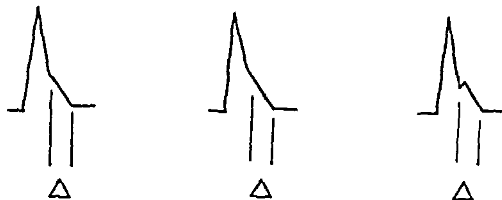


Fig 5 Different configurations of "terminal delta waves" with the durations indicated

but he did not further investigate whether these "terminal delta waves" were a manifestation of pre excitation like the *initial* ones

That there may be a connection between such terminal delta waves and pre excitation is clear from cases such as in fig 4 where a terminal delta wave appears when *the initial* one disappears Hence it was considered important to study whether terminal delta waves are a manifestation of pre excitation

The aim of this study was to determine whether there is an over representation of terminal delta waves in groups of previously diagnosed pre excitation and paroxysmal tachycardia each compared with one control group

Material

From the 26 subjects with previously diagnosed pre excitation (p 11) 6 fulfilled my criteria for pre excitation as did 6 from the 31 subjects with paroxysmal tachycardia (p 15) The electrocardiograms of the remaining 20 pre excitation subjects the 25 tachycardia patients and their controls were used for this study

Methods

The leads studied were I II III, CR and V_1 2 4 5 and 7 and the criteria for a terminal delta wave were 1 that the down stroke of the R wave or the upstroke of the S wave should consist of two parts with different slopes the latter of which should be less steep (fig 5) 2 that the delta wave should occur in two or more leads the CR and V leads being considered equal. The duration of the terminal delta waves was measured as the average of two complexes in the lead where they were most conspicuous

Results

As can be seen in tables 7 and 8 there was no significant difference between the pre excitation material and the controls either in regard to terminal delta waves of ≥ 2 ≥ 3 ≥ 4 or ≥ 5 csec Nor were there any significant differences between the cases with paroxysmal tachycardia and their controls

Discussion

An alternation from an initial to a terminal delta wave could be explained as a

	Pre excitation cases	
	with	*without*
	paroxysmal tachycardia at the beginning of the observation period	
Not traced	4	2
Dead — before the original publication	6	7
Dead — during the observation period	11	
Not re examined	2	2
Re examined	31	10
	54	21

Table 9 Survey in 1965 of 75 pre excitation cases published by Öhnell and Biorck in 1944 and 1946 respectively (34 8)

examination about attacks of tachycardia and two gave a history of paroxysmal tachycardia which had probably occurred as early as at the time of Öhnell s examinations The total number of patients with paroxysmal tachycardia at the time of Öhnell s and Biorck s examinations is thus 54 Of these four were among those who could not be found leaving 50 patients for the follow up study

Mortality

Of the 50 patients with paroxysmal tachycardia who could be traced 6 were already dead when the original series were published (Ö 3 11 18 23 26 and 34) and 11 had died during the observation period 1944—1965 The causes of death as on the death certificates are presented in table 10

(The causes of death in the 7 deceased among those who did not have paroxysmal tachycardia at the beginning of the observation period are presented in Appendix V)

As far as is known, only one patient died during an attack of tachycardia (Ö 11)

Comments

The non specific diagnosis of organic heart disease (Ö 23 34 57) might as late as in the 1940 s have been the expression for an undiagnosed pre-excitation (paroxysmal tachycardia electrocardiographic changes and/or heart murmur (6) since childhood) Chronic myocarditis (Ö 3 70) was probably a frequent diagnosis in obscure cardiac cases still in the 1940 s In the autopsied case (Ö 3) however the diagnosis was supported by the findings of large amounts of small round cell foci and transformation to connective tissue in the myocardium (34) The diagnosis of myocarditis in case Ö 11 was based on moderate

STUDIES ON PROGNOSIS

The prognosis in pre excitation is generally reported to be good, unless associated with frequent or prolonged attacks of tachycardia (5, 15, 22, 23, 26, 59) This reservation is based on reports of isolated cases of sudden death in connection with an attack of tachycardia (26, 34, 45) American insurance companies are said to consider the mortality increased by 25—100 % in the W P W anomaly and 60—300 % in the W P W syndrome (17) while neither frequent nor prolonged attacks of tachycardia cause a premium increase for a subject with the W P W syndrome in Sweden (53) The reports on the prognosis of pre excitation are thus varying and they seem in no case to be based on systematic follow up studies with long observation times

There were two Swedish series available for a follow up study with reference to the long term prognosis in pre excitation, Öhnell's series published in 1944 (34), and Biorck's in 1946 (8), together comprising 75 cases A study of the prognosis is best based on those subjects of these 75 who had sought medical advice because of symptoms of pre excitation i e paroxysmal tachycardia It is difficult now to decide who sought medical advice because of paroxysmal tachycardia and who did for other disorders It is reasonable, however to assume that all those who have had repeated attacks of tachycardia of fairly long duration find them so embarrassing or alarming that they seek medical advice Therefore this study of the prognosis was based on the patients who had had attacks of tachycardia already at the time of Öhnell's and Biorck's examinations In these patients the prognosis was studied with reference to a) mortality, b) paroxysmal tachycardia, c) ECG changes, d) angina pectoris, and e) cardiac insufficiency At the same time the concurrence of f) valvular heart disease and g) cardiomyopathy was studied

Material

Öhnell had collected the 70 patients during his routine work at an electrocardiographic laboratory and through a search of the archives of several hospitals in Stockholm and the provinces and he also obtained some patients directly from colleagues Biorck had found his 5 patients in a department of medicine

Öhnell's patients were located with the assistance of the population registers, his notes and electrocardiograms In 5 cases these were too incomplete for identification however Biorck's patients were identified through the patients records at Sabbatsbergs Hospital One of these patients proved to be a foreigner and impossible to find Altogether 69 of the 75 patients (92 %) could be found in the population registers (table 9)

According to Öhnell's and Biorck's findings 52 of the 75 patients had paroxysmal tachycardia In 5 other of Öhnell's cases there was some uncertainty as regards paroxysmal tachycardia Of these 5 patients all except one could be asked at this re

	Pre excitation cases	
	with	*without*
	paroxysmal tachycardia at the beginning of the observation period	
Not traced	4	2
Dead — before the original publication	6	7
Dead — *during the observation* period	11	
Not re examined	2	2
Re examined	31	10
	54	21

Table 9 Survey in 1965 of 75 pre-excitation cases published by Öhnell and Biorck in 1944 and 1946 respectively (34 8)

examination about attacks of tachycardia and two gave a history of paroxysmal tachy*cardia which had probably occurred as early* as at the time of Öhnell s examinations The *total number of patients with paroxysmal* tachycardia at the time of Öhnell s and Biorck s examinations is thus 54 Of these four were among those who could not be found leaving 50 patien s for the follow up study

Mortality

Of the 50 patients with paroxysmal tachycardia who could be traced 6 were already dead when the original series were published (Ö 3 11 18 23 26 and 34) and 11 had died during the observation period 1944—1965 The causes of death as on the death certificates are presented in table 10

(The causes of death *in the 7 deceased* among those who did not have paroxysmal tachycardia at the beginning of the observation period are presented in Appendix V)

As far as is known only one patient died during an attack of tachycardia (Ö 11)

Comments

The non specific diagnosis of organic *heart disease (Ö 23 34 57) might* as late as in the 1940 s have been the expression for an undiagnosed pre-excitation (paroxysmal tachycardia electrocardiographic changes and/or heart murmur (6) since childhood) Chronic myocarditis (Ö 3 70) was probably a frequent diagnosis *in obscure* cardiac cases still in the 1940 s In the autopsied case (Ö 3) *however the diagnosis* was supported by the findings of large amounts of small *round cell* foci and transformation to connective tissue in the myo*cardium (34) The diagnosis* of myocarditis in case Ö 11 was based on moderate

Case no	Sex	Age at death	Diagnosis on the death certificate	Autopsy
Ö 3	F	43	*Chronic myocarditis*	+
Ö 7	F	62	Chronic nephritis	
Ö11	F	32	Myocarditis	+
Ö18	M	40	*Suicide*	
Ö20	F	57	LED	
Ö23	M	21	Organic heart disease	
Ö26	F	70	Cancer of the stomach	
Ö28	M	31	Suicide	
Ö30	M	27	Chronic nephritis	
Ö31	M	61	Myocardial infarction	
Ö34	F	62	*Organic heart disease*	
Ö45	M	47	Pulmonary tuberculosis	+
Ö47	M	80	Fibrosarcoma	
Ö48	M	61	*Cardiac insufficiency*	
Ö51	M	66	Mitral valvular disease	+
Ö57	M	61	Organic heart disease	
Ö70	M	55	*Chronic myocarditis*	

Table 10 The ages and diagnoses on the death certificates of the 17 deceased among 50 subjects with pre excitation and paroxysmal tachycardia Ö 1 etc refers to the case numbers in Öhnell s report (34)

oedema of the myocardium However this may also have been caused by the prolonged attack of tachycardia which led to death

Comparison with mortality in Sweden

The 44 patients who were still alive at the time of the original publications were compared to the general population with regard to the mortality during the follow up period *Although not published until 1946* Biörck s two cases among the 44 above had been hospitalized in 1941—1944 A comparison could therefore be made for the period 1945—1965 for all cases Of these 44 patients 29 were males and 15 *females In* 1945 the ages of the males varied between 9 and 59 years and of the females between 25 and 63 years

The expected mortality during the follow up period was calculated *from* tables of mortality rates in Sweden given as means for five year periods for each age and sex (52) The probability of surviving the next year was calculated by subtracting the mortality rate from 1 The product of 21 consecutive such probabilities was the probability for a subject to be alive at the end of the follow up period The sum of 44 such probabilities was the expected number of persons alive at the end of the observation period In this way the expected mortality during the observation period was found to be 7 6 which does not differ significantly from that observed 11 ($P > 0\ 1$ x^2)

Comments

Even if all the 4 patients with paroxysmal tachycardia since the beginning of the follow up *period* who *could not* be located, were dead this would not have changed the

result into an over mortality for the pre excitation group

There is an over representation of city inhabitants in the pre excitation group as compared with the general population and in the general population there exists an over mortality among city dwellers as compared to rural dwellers This could lead to an over mortality for the pre excitation group in comparison to the general population which however has not been observed

Thus there is no over mortality for these pre excitation subjects with a 21 year follow up period in spite of all having had attacks of tachycardia which in some had been frequent This finding confirms the general opinion in literature (5 15 22, 23 26) of a good prognosis in pre excitation It does not support the usual reservations made for cases with frequent attacks of tachycardia

The re-examination

Material

Of the 50 pre excitation patients with paroxysmal tachycardia at the beginning of the observation period 33 were alive in 1965 (table 9) 20 males and 13 females 29—73 and 46—84 years old respectively Of these 33 31 (94%) could be re examined Thirty were examined by the author in 1965 21 at Serafimerlasarettet and 9 at hospitals in the provinces Another one living abroad was investigated by her answering a postal questionnaire and sending an electrocardiogram and a chest X ray both taken in 1965 Two patients could not be re examined due to lack of co-operation

Furthermore 10 of the 12 pre excitation subjects who had not had tachycardia at the time of the original examination and who were still alive were re examined (table 9)

Methods

The examination included a history a physical examination an electrocardiogram and a chest X ray The history was recorded according to a questionnaire with reference to 1) paroxysmal tachycardia (repeated attacks of very rapid palpitations regular or irregular of sudden onset) 2) angina pectoris (attacks of pain or oppression in the chest on effort or excitement which forced the patient to relative rest lasting 15 minutes at the most and unassociated with attacks of tachycardia) 3) cardiac insufficiency (dyspnea at rest paroxysmal nocturnal dyspnea or breathlessness when walking with ordinary speed on even ground in the absence of chronic respiratory disease and unassociated with attacks of tachycardia) and 4) cardiac medication

A detailed physical examination of the cardiovascular system was performed Pathological findings at cardiac auscultation were recorded on a phonocardiogram

Electrocardiograms were recorded and examined as described on p 9 Electrocardiograms without a pre excitation pattern were interpreted according to Goldman (17)

In cases without delta waves attempts were made to provoke delta waves (34 40) by prolonged maximal exspiration and inspiration carotid sinus pressure and finally a bicycle ergometer test (46) Exercise registrations were usually performed every minute during work and up to 10 minutes after work, the 6 minutes load being 600 kpm/min for males and 400 kpm/min for females

The heart volume was calculated from chest X ray films taken in the erect position The upper normal limit was set at 530 ml/

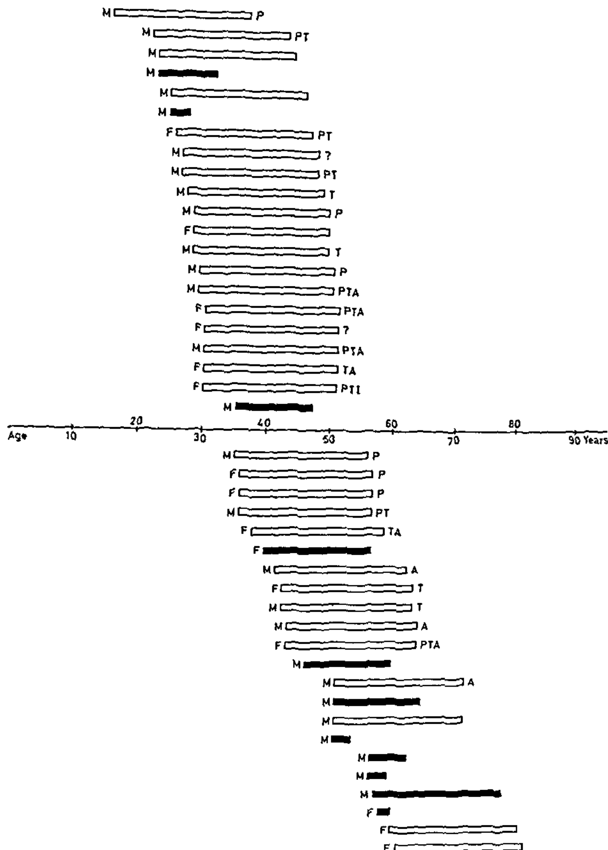


Fig 6 The course during 21 years of the 44 patients with pre excitation electrocardiograms and paroxysmal tachycardia M = male F = female filled symbols = dead open symbols = alive P = pre excitation electrocardiogram at the follow up examination T = attacks of tachycardia during the last year, A = effort angina I = cardiac insufficiency

No attacks for several years	14 (45 %)
No attack during the last year	2 (6 %)
A few attacks each year	4 (13 %)
One or two attacks each month	7 (23 %)
One or two attacks each week	4 (13 %)

Table 11 Rates of attacks of tachycardia in 31 re-examined pre excitation patients

square metre BSA for males and at 170 ml/square metre BSA for females (M+2 5 S D) (32)

Results

The results are presented in Appendix I and fig 6

In several patients an early systolic heart murmur of medium frequency was heard along the left sternal border which was considered clinically non significant and was not listed in Appendix I if not exceeding degree 2/6

The blood pressure measured in the supine position has been included in Appendix I only when the diastolic pressure (point of disapperance) was 100 mm Hg or more

The results will be summed up and discussed on the following pages under the headings of Paroxysmal tachycardia Electrocardiograms Angina pectoris Cardiac insufficiency Valvular heart disease and Cardiomyopathy

No follow up study seems to exist with which these results could be compared

Paroxysmal tachycardia

The diagnostic criterion for paroxysmal tachycardia has been repeated attacks of very rapid palpitations with sudden onset

Of the 31 re examined patients with paroxysmal tachycardia at the beginning of the observation period 17 (55 %) reported that the frequency of attacks had decreased during these years while 5 (16 %) thought that they had become more frequent (Ö 14 32, 41 67 and B 2) Four (13 %) had not noticed any change (Ö 6 10 65 and 68) and in 5 cases (16 %) their statements were uncertain or unavailable (Ö 13 38 50 53 and 60)

The frequency of attacks at present is shown in table 11

The majority of the patients reported a decrease through the years in the frequency of attacks of tachycardia This would suggest a decrease of attacks with age However the patients without any attack during the last year were not older than those with attacks ($P > 0 1$ x^2)

Nor did the patients with and without attacks of tachycardia during the last year differ as regards the frequency of normalized electrocardiograms ($P > 0 1$ x^2) (table 12)

Two patients (Ö 36 and 41) stated that they had two kinds of attacks of tachycardia, one with regular rhythm the other being irregular Both paroxysmal atrial fibrillation and flutter have been described to occur in pre excitation (1 13 15 22 35 48 56 58) and either could explain their irregular palpitations

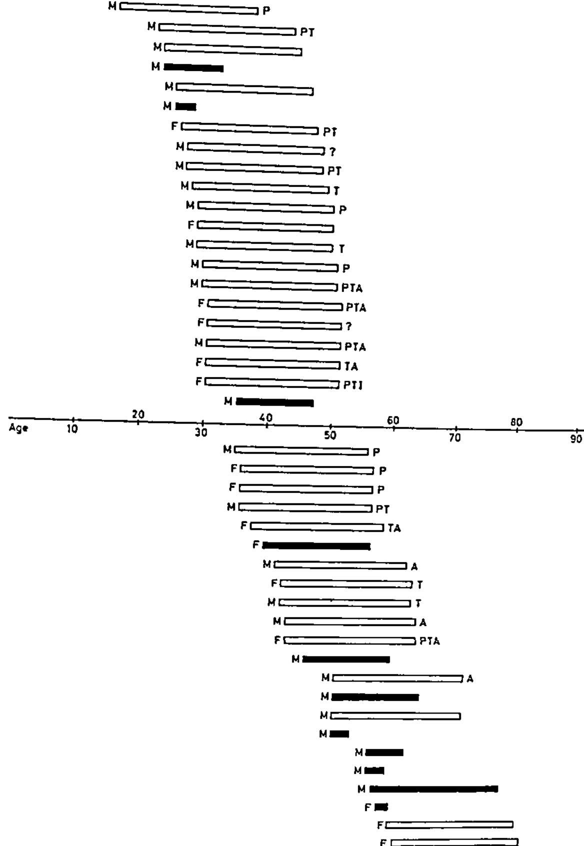


Fig 6 The course during 21 years of the 44 patients with pre-excitation electrocardiograms and paroxysmal tachycardia M = male F = female filled symbols = dead open symbols = alive, P = pre-excitation electrocardiogram at the follow up examination T = attacks of tachycardia during the last year A = effort angina, I = cardiac insufficiency

S-T segment occurred in 5 cases (Ö 53 55, 56 68 B 3) but could be explained by digitalis in three of them (Ö 55 56 and 68) The two other patients were both 64 years old

Angina pectoris

The diagnostic criteria for effort angina were attacks of pain or oppression in the chest on *effort or excitement* which force the patient to relative rest and last for 15 minutes at the most The attacks had to occur without any connection with attacks of tachycardia

Of the 31 re examined patients with paroxysmal tachycardia at the beginning of the follow up period 7 (23 %) fulfilled these criteria for effort angina (Ö 6 9 10 16 42 65 and 66) Of these 7, 5 were males 50—73 years old and 2 females both 51 years old Three had a pre excitation electrocardiogram and four had not None of the four normalized electrocardiograms showed ST J depressions None had a heart murmur indicative of aortic valvular disease or an obstructive cardiomyopathy as the cause of the effort angina One of the 7 took nitroglycerine Besides these 7 with *effort angina one woman 63 years old* (Ö 14) gave a history of nocturnal angina

Effort angina in 23 % of the patients seems to be a remarkably high frequency and it could not in any patient be explained as coronary ischemia in connection with attacks of tachycardia nor by angina pectoris as having been the reason for the original examination The controls from a postal inquiry examination of twins (14) were also chosen as the controls for this study These 857 controls (= 81 % participation) were selected at random from the entire population of Sweden in the age group 40—79 Sending the same questionnaire to the pre excitation patients enabled an identical interview technique for both groups The criteria for effort angina were slightly different in this inquiry The alternative to chest pain was not oppression but discomfort and the duration of the pain was limited to 10 minutes

The questionnaire from the twin examination was sent to the 7 patients with effort angina some months after re examination In all 7 cases the answers fulfilled the criteria for effort angina from the twin examination The frequency of angina pectoris in the control material was (14)

Age years	*Male* %	*Female* %
40—49	4 8	6 1
50—59	10 2	8 5
60—69	*9 6*	*8 6*
70—79	14 0	11 1

From these figures a geometric mean was calculated according to the distribution of sex and age in the re examined pre excitation patients This mean of the control group 8 2 % was compared to the frequency of effort angina in the pre excitation group The comparison showed an over representation of effort angina in the pre excitation group ($P < 0.05$ χ^2 test) This probable over morbidity in effort angina cannot be explained on the basis of the present study

The frequency of myocardial *infarction* in this series has not been studied

Cardiac insufficiency

The results here are based on the 31 re examined patients who have had paroxysmal

Patients with	pre excitation electrocardiogram	normalized electrocardiogram
who have had attacks of tachycardia during the last year	9	6
who have had no attacks of tachycardia during the last year	6	10

Table 12 The occurrence of pre excitation electrocardiogram and a history of attacks of tachycardia during the last year at *re examination* of 31 pre excitation patients

Of the 11 subjects without paroxysmal tachycardia at the beginning of the follow up period, 5 had suffered such attacks later on The attacks of tachycardia had probably begun when the patients were in their twenties or thirties

Electrocardiograms

Of the 31 re examined patients with paroxysmal tachycardia at the beginning of the observation period 7 fulfilled Wolff Parkinson White s or Öhnell s configurational criteria at re examination (Ö 13 11, 32, 37 41 42, 51) and one patient showed intermittent genuine normalization (Ö 9) The conventional criteria therefore provided a diagnosis for 8 patients out of 31 (26 %) The criteria used here (a delta wave in at least two leads and with a duration of 3 csec or more in at least one of them) were fulfilled by 15 electrocardiograms (48 %) The electrocardiograms of the other 16 patients had normalized at re examination

The normalization of pre excitation electrocardiograms could be an effect of age among other factors but the patients with a normalized electrocardiogram at re examination were not significantly older than those with pre excitation (P > 0 05 χ^2)

Of the 16 patients without a pre excitation electrocardiogram at re examination all except two (Ö 55 and 60) were subjected to a provocation test by prolonged maximal inspiration and exspiration but this did not in any case result in a delta wave of 3 csec Carotid sinus pressure was performed on all patients except one (Ö 60) It was usually effective as it decreased the heart rate, but did not result in a diagnosis of pre excitation in any patient Bicycle work was performed by 4 patients (Ö 10 16, 36 65) but no delta waves occurred neither during work nor after Sandberg (40) was successful in abolishing normalization by an exercise test in two of 28 pre excitation subjects The frequency of 0 out of 4 here does not differ from that

The 16 electrocardiograms without a pre excitation pattern were also examined in other respects Three cases of left axis deviation (< —30°) (Ö 55 56 and 66) were observed These patients were 65 83 and 84 years old respectively ST J depressions in the left precordial leads of at least 0 5 mm with a horizontal or downward sloping

S-T segment occurred n 5 cases (Ö 53 55 56 68 B 3) but could be explained by d g tal s in three of them (Ö 55 56 and 68) The two other pat ents were both 64 years old

Angina pectoris

The d agnost c cr teria for effort ang na were attacks of pain or oppress on n the chest on effort or excitement which force the pat ent to relative rest and last for 15 m nu es at the most The attacks had to occur w thout any connection w th attacks of tachycard a

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Send ng the san e questio ina re to the pre excitat on pat ents enabled an dent cal in terv ew techn que *for* bo h groups The cr ter a for effort ang na were sl ghtly d f ferent n th s inqu ry The alternat ve to chest pa n was not oppress on but d scom fort and the durat on of the pa n was l m t ed to *10 m nutes*

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70—79	14 0	11 1

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The frequency of myocard al nfarction n th s ser es has not been studied

Card ac insuffic ency

The results here are based on the 31 re examined patients who have had paroxysmal

tachycardia at the beginning of the observation period

One patient (3 %) was probably in heart failure with effort dyspnea and cardiac enlargement, and she was on digitalis (B 2) Cardiomyopathy may be the cause of this insufficiency (see p 31)

Another 11 patients (36 %) had symptoms or signs suggestive of cardiac insufficiency Two of these gave a history of effort dyspnea and three others of paroxysmal nocturnal dyspnea At examination one more patient exhibited effort dyspnea while another had dyspnea at rest Still another was cyanotic Three others showed a cardiac enlargement on X ray However, none of these 11 had a combination of symptoms and findings Four of them were digitalised (Ö 12 51 56 and 68) and if these are considered to be in heart failure the total occurrence of cardiac insufficiency among these 31 patients is 5 (16 %) Two were males aged 19 and 51 years respectively and three were females the ages varying between 51 and 83 years The electrocardiogram showed pre excitation for three of them

Of the 11 who had died during the follow up period one had the diagnosis of cardiac insufficiency on the death certificate (Ö 19)

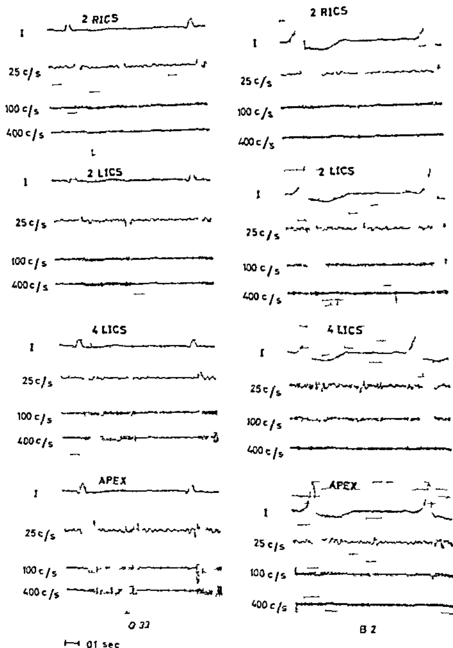
As regards cardiac insufficiency no suitable control material was available

Valvular heart disease

Two of the 31 re examined patients with paroxysmal tachycardia at the beginning of the follow up period had pathological findings on cardiac auscultation at re examination A male of 45 (Ö 33) had a pansystolic murmur of high frequency best heard over the apex degree 4/6 and poorly conducted towards the axilla (fig 7) The second heart sound was accentuated at the second right intercostal space, the blood pressure was 200/100, the femoral pulses being ordinary This patient s electrocardiogram did not show a pre excitation pattern and was also normal in other aspects apart from a ST J depression of 0 5 mm with an upward sloping S T segment in lead CR_4 The heart volume was normal but the left ventricle was considered to be relatively enlarged The man had no cardiac symptoms whatsoever These findings would agree with slight mitral incompetence (or possibly a small ventricular septal defect) Further investigations have been deferred in the absence of symptoms

The other patient was a 51 year old woman (B 2) with an atrial sound and a short early systolic murmur of medium frequency over the third left intercostal space degree 3/6 (fig 7) This patient suffered from effort dyspnea and she was digitalised An X ray of the heart and lungs showed a slight cardiac enlargement mainly affecting the left ventricle This case had previously been regarded as having mitral stenosis and incompetence (8) At this examination no diastolic murmur or opening snap could be heard or recorded and there was no left atrial enlargement on X ray The systolic murmur could be physiological or caused by the pre excitation as shown by Bergland et al (6) in a subject with intermittent pre excitation The effort dyspnea, the atrial sound and the cardiac enlargement remain to be accounted for however and this case will be discussed further under the heading of Cardiomyopathy (p 34)

Therefore among those re examined there was one case of valvular heart disease (or ventricular septal defect) (Ö 33) Among



F g 7 Phonocard og ams from the two patients w th patholog cal find ngs at card ac auscultat on

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Of the 11 who had died during the follow up period one had the diagnosis of cardiac insufficiency on the death certificate (Ö 18)

As regards cardiac insufficiency no suitable control material was available

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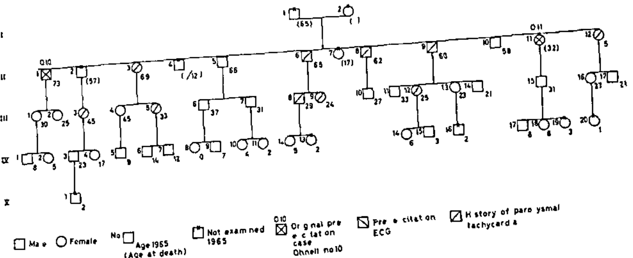


Fig 8 Pedigree of family B

tachycardia at the beginning of the observation period

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Of the 11 who had died during the follow up period one had the diagnosis of cardiac insufficiency on the death certificate (Ö 18).

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Therefore among those re examined there was one case of valvular heart disease (or ventricular septal defect) (Ö 33). Among

Summing up among 50 patients with paroxysmal tachycardia at the beginning of the follow up period there was one probable case of cardiomyopathy among those, who had died and one possible case among those re examined In the absence of a suitable control material it was impossible to decide whether this frequency represents an over morbidity or not

Comments

Thus of the 31 re examined pre excitation patients with paroxysmal tachycardia at the beginning of the follow up period 9 (29%) had another probable cardiac disease such as effort angina, cardiac insufficiency or valvular heart disease No patient had more than one of these additional conditions and in 11 (36%) the examination revealed nothing abnormal

those autopsied there was one case with the diagnosis of mitral valvular disease (Ö 51) Together this makes two cases of valvular heart disease out of the 50 patients with paroxysmal tachycardia In the absence of a suitable control material it was impossible to decide whether this frequency represents an over morbidity or not

Cardiomyopathy

Cardiomyopathy is used in the sense of primary idiopathic chronic myocardial disease in the present study The cardiomyopathy may be obstructive or non obstructive In both forms pre excitation sometimes occurs (9 10, 11 50 55)

Obstructive cardiomyopathy

Common symptoms and physical signs of obstructive cardiomyopathy (subaortic stenosis caused by asymmetrical hypertrophy) include dyspnea an atrial sound a systolic murmur of somewhat late onset maximal at the apex, and an enlargement of the left ventricle on X ray (7 10 18)

Among the re examined patients there was one case with findings suggestive of obstructive cardiomyopathy The previously mentioned woman with the systolic murmur and the atrial sound (B 2) had effort dyspnea and also an enlarged left ventricle on X ray This might be compatible with an obstructive cardiomyopathy but her murmur was early systolic and short The patient declined further investigations

Obstructive cardiomyopathy may also be a post mortem diagnosis but in the autopsy reports of those deceased there is no mention of a localized hypertrophy of the ventricular septum

Non obstructive cardiomyopathy

Patients with non obstructive cardiomyopathy sometimes give a family history of cardiac disease or sudden death at an early age This form of cardiomyopathy usually appears before the age of 50 as non symptomatic cardiac enlargement, Adams Stokes attacks or heart failure which then usually is biventricular already at the onset Emboli to the pulmonary and systemic circulations often occur during the course of the disease which does not infrequently terminate in sudden death (11, 16 18, 30, 31, 51)

Among those re examined there was no patient with Adams Stokes attacks but four patients had cardiac enlargement (Ö 2 56 B 2 and 3) Only in one case the previously mentioned woman (B 2) the cardiac enlargement could be traced back, with the assistance of old X rays to the age of 40 where the possibility of coronary heart disease as the cause of heart enlargement is fairly small in a female This patient was also the only one in heart failure but this was not biventricular The case is thus neither typical of non obstructive nor of obstructive cardiomyopathy but cardiomegaly from the age of 40 dyspnea on effort and an atrial sound in absence of significant murmurs and hypertension favours a diagnosis of chronic myocardial disease As the patient declined further investigations her myocardial disease could not be classed as primary and idiopathic

Of those autopsied one patient with sudden death at the age of 43 (Ö 3) would probably to day have been diagnosed as cardiomyopathy No changes in the heart were observed macroscopically but there was generalized round cell infiltration and fibrosis microscopically

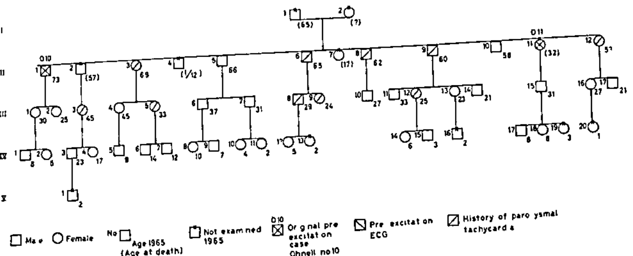


Fig 8 Pedigree of family B

STUDIES ON HEREDITY

Pre excitation has been described as occurring among siblings (e g 3, 33, 13) and parent child combinations (e g 20, 29, 55) Harnischfeger (20) recorded pre excitation electrocardiograms in a father, his son, and among the grandchildren, in two identical twins and one fraternal twin These reports support pre excitation being an inherited anomaly On the other hand, Warner and McKusick (51) examined 80 members of 14 W P W families without finding any additional cases Nor could they establish any increase of consanguinous matings among the parents of these W P W cases Thus, earlier investigations have given diverging results regarding the heredity of pre excitation

Öhnell (33) described two families (figs 8 9) where some siblings (B II 1/3/6/9/11 S K III 26/27/28/30) had possible or probable pre excitation electrocardiograms and attacks of tachycardia In one of these siblings a woman of 32 (B II 11) he found a subepicardial muscle bundle at autopsy which connected the left atrium and the left ventricle dorsally (34) Öhnell thought that these findings taken together, were in favour for pre excitation being an inherited anomaly

Twenty five years have passed since Öhnell examined these two families which now include two or three more generations (B III IV and V S K IV and V) This renders possible a study on the heredity in pre excitation

Material

The two families were identified and traced with the aid of Öhnell s notes and the Swedish population registers Most of the members of the two families turned out to be living in Stockholm and its immediate surroundings In 1965 both families were spread over four generations, originally including 198 members, 50 in family B and 148 in family S K (figs 8 and 9) Three of these (B II 1 B II 11 S K III 30) formed part of Öhnell s 70 pre excitation subjects (34) Of the 198 9 were dead among them one of the three original cases (B II 11) Of the 189 still alive in 1965 all were called in for examination except 20 children who were under four years of age, and 8 children who did not live together with their parents These 28 children were not called as it was considered psychologically unwise Of the 161 invited for examination 148 attended and so did 4 children under 4 who had not been called altogether 152 persons (= 100 % participation in family B 89 % in family S K)

Methods

The examination was carried out in 1965 For the majority of the family members it took place at Serafimerlasarettet and for the rest at other hospitals The examination consisted of 1) taking a history with reference to paroxysmal tachycardia (repeated attacks of very rapid palpitation of sudden onset)

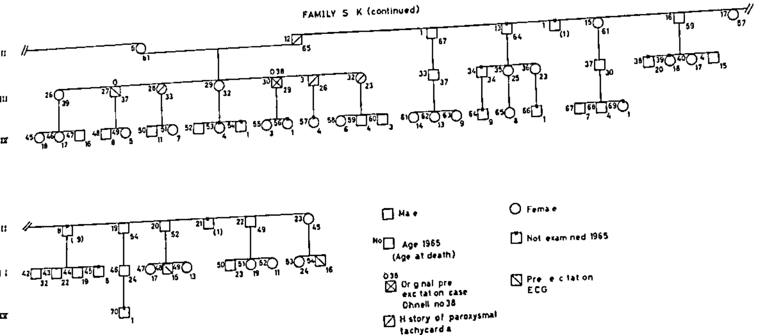
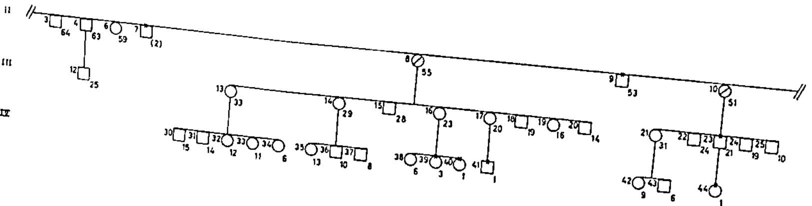


Fig. 9 Pedigree of family S K

FAMILY S K



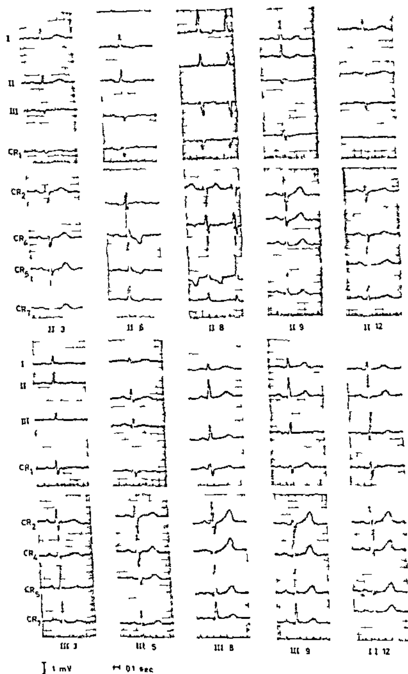


Fig 10 Electrocardiograms from all the members of family B who have pre excitation or *paroxysmal tachycardia*

For the subjects in outlying areas this was done by letter 2) an electrocardiogram which, both at Serafimerlasarettet and at the other hospitals, was recorded with a 3 or 4 channel ink writing apparatus (Mingograph, Elema Schonander, Stockholm) Leads I, II, III, V_1, V_4, V_7 were recorded in all and at Serafimerlasarettet also leads V_2, V_5, CR_1, CR_2, CR_4, CR_5 and CR_7, the paper speed being 50 mm/sec. The electrocardiograms were scrutinized with reference to delta waves in these leads When a delta wave fulfilling the configurational criterion on p 9 occurred in at least two leads and had a duration of 3 csec or more in one of them the case was regarded as one of pre excitation The reproduced electrocardiograms from Öhnell s examination (33) of the brothers and sisters B II 1/5/6/9/11 and S K III 26—30 were also examined according to these pre excitation criteria

Bearing the possibility in mind that heterozygosity might reveal itself in the form of delta waves shorter than 3 csec the frequency of delta waves of 2 csec was also studied (delta waves of 1 csec being difficult to discern) For each family member of 16 years or more a control of the same sex and age ± 3 years was selected at random from a general health examination of civil servants at Serafimerlasarettet in 1965 The electrocardiograms of these controls were also examined as regards delta waves of 2 csec No controls aged 3—15 were available

The parents of those with a pre excitation electrocardiogram at Öhnell s or this examination or with a history of paroxysmal tachycardia were asked if they knew about any consanguinity If the parents were dead the question was put to the subject himself

Results

At the examination of the electrocardiograms reproduced in Öhnell s report (33), all the three original cases (B II, 1, B II, 11, S K III, 30) were found to fulfil the pre excitation criteria used here, as was also a brother of the original case in family S-K (S K III 27)

At this examination of family B neither the only one alive of the two original subjects (B II 1), nor any of the other 40 examined members showed a pre-excitation electrocardiogram In family S-K, too both subjects with a pre excitation electrocardiogram at Öhnell s examination (S K III 27 S-K III, 30) were now found to show normalization, but two boys of this family, 15 and 16 years old (S K III 48 S K III, 54) showed pre excitation These four pre excitation subjects were first cousins

Seven of the 89 members of families B and S K who were 16 years or more had a delta wave of 2 csec (B II 12, B III, 15, S K III 5, S K III 26 S-K III 27, S K III 28 S K IV 21) Compared to 3 such cases from the control group this shows no significant difference ($P > 0.1$ x^2)

Nineteen members of families B and S-K had a history of paroxysmal tachycardia including two of the three examined pre excitation subjects who were diagnosed on Öhnell s electrocardiograms (33) Among these 19 there were four parent child combinations (B II 3 — III 5 B II 6 — III 8/9 B II 9 — III 12 S K II 12 — III 29/30/31/32) The occurrence of paroxysmal tachycardia was independent of the consecutive order of the siblings

Consanguinity of the parents was not known for any subject with a pre excitation electrocardiogram or paroxysmal tachycardia

For further details see figs 8 9 10 11

Discussion

The pedigrees (figs 8, 9) were based on information from the population registers When checked with the assistance of the family members at the time of examination occasional additions were made as children born out of wedlock or by previous marriages are difficult to trace

Pre excitation At this single examination only two more pre excitation cases were found in these two families At the same time of the 4 subjects who had shown a pre excitation electrocardiogram at Öhnell s examination the three examined showed a normalized pattern That pre excitation is a changing condition is also evident from the finding that 20 of Öhnell s 41 cases had normalized on re examination (p 10) In other words a person may be affected in spite of a normal electrocardiogram. Under these circumstances the findings at these two examinations do not permit the conclusion that pre-excitation is an inherited anomaly in families B and S-K

Paroxysmal tachycardia Families with several members with paroxysmal tachycardia (e g 2 39 55) have been reported with and without associated pre excitation Lundberg (28), on the other hand, found only one case of paroxysmal tachycardia among the parents of 44 infants with attacks of tachycardia About one half of these infants had pre excitation as well Electrocardiograms were not recorded in the parents

In all cases of paroxysmal tachycardia in families B and S-K every parent examined was also found to be affected (B III 5 — II 3 B III 8/9 — II 6 B II 12 — II 9 S K III 28/30/31/32 — II 12) This speaks in favour of autosomal dominant inheritance of paroxysmal tachycardia in these families (38) A sex linked X chromosomal dominant and recessive inheritance respectively are inconsistent with a father son combination in both families (38)

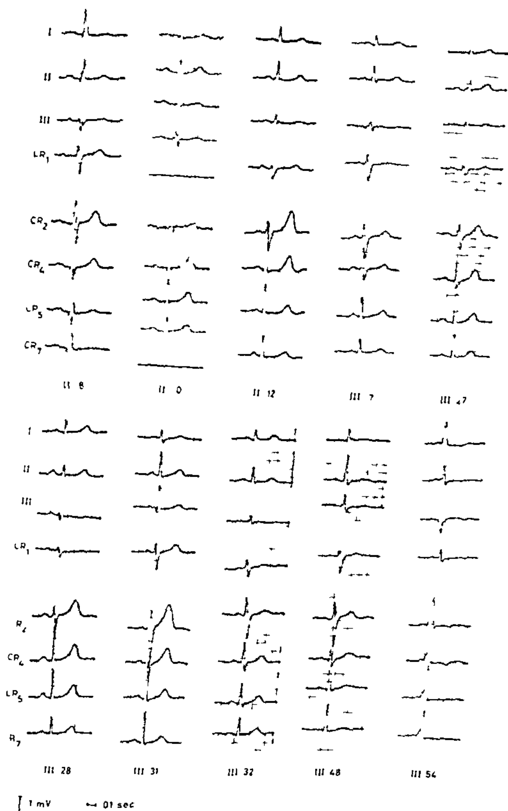


Fig 11 Electrocardiograms from all the members of family S-K who have pre excitation or paroxysmal tachycardia

Discussion

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SUMMARY

The present study is concerned with the diagnosis, prognosis and heredity of pre excitation

Studies on the electrocardiographic criteria

At a re examination of 41 subjects with pre excitation, published by Öhnell in 1944 (34) and Biorck in 1946 (8) the electro cardiograms fulfilled Wolff Parkinson White s or Öhnell s criteria for pre excitation in only 11, though several more had delta waves The question then arose whether a delta wave without a co existing short P R interval is sufficient for a diagnosis of pre excitation without the support of ancillary criteria such as genuine normalization and the concertina phenomenon Furthermore the question arose as to how short a delta wave may be without losing its significance as regards pre excitation

In order to find the answers to these questions comparisons of the frequency of delta waves without co existing short P R interval were made between the previously diagnosed pre excitation subjects and controls on the one hand and between paroxysmal tachycardia patients and controls on the other The criteria for a delta wave were 1) that the upstroke of the R wave or the downstroke of the QS wave should consist of two parts with different slope of which the first should be less steep 2) that it should occur in at least two of the studied leads I II III CR or $V_{1\ 2\ 4\ 5}$ and 7 3) that it should start before the QRS complexes in the leads without a delta wave In the pre excitation as well as in the paroxysmal tachycardia cases there was an over representation of delta waves with the duration of 3 csec [1] or more

If such delta waves were accepted as the sole criteria for a pre excitation electrocardiogram, this could lead to false positive diagnoses However, the *maximum* number of possibly false positive diagnoses with these criteria was found to be essentially equal to the *actual* number of false negative diagnoses with the conventional criteria The real risk of false positive diagnoses is probably still smaller in the clinical situation where pre excitation has its main importance i e in patients with attacks of tachycardia, as paroxysmal tachycardia is over represented among subjects with pre excitation The frequency of paroxysmal tachycardia was not significantly different in subjects diagnosed with the conventional pre excitation criteria and in those diagnosed with the criteria discussed here Consequently the following criteria for pre excitation were found to be clinically advantageous delta waves in at least two leads with a duration of 3 csec or more in at least one of them (independent of the P R interval) Four electrocardiograms out of 2300 from a general health survey fulfilled these criteria (2 ‰) and 19 out of 5000 from a hospital archives (4 ‰) In the literature no similar study was found with which these results could be compared

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[1] 1 csec = 0 01 sec

Öhnell (33) found a gradually descending terminal portion of the QRS complex in some relatives of subjects with pre excitation In this study two electrocardiograms are presented on p 21 *in which such* terminal delta waves alternate with initial ones These two observations imply an association between pre excitation and terminal delta waves In order to study this a comparison was made between *the pre*viously diagnosed pre excitation subjects and controls and between patients with paroxysmal tachycardia and controls with regard to the occurrence of delta waves at the end of the QRS complex No *significant* differences were found and this contradicts the hypothesis that a terminal delta wave is a manifestation of pre excitation In the literature no similar study was found with which these results could be compared

Studies on prognosis

The prognosis in pre excitation was studied *with reference to mortality paroxysmal* tachycardia angina pectoris and cardiac insufficiency At the same time the concurrence of valvular heart disease or cardiomyopathy with pre excitation was studied These studies were based on the 50 patients from Öhnell s (34) and Biorck s (8) series who had symptoms of pre excitation i e paroxysmal tachycardia already at the original examination more than 21 years previously Of these 50 patients 6 had died before the series were published 21 years ago During the follow up period 11 *more* died which compared with the expected mortality of 7 6 cases did not constitute a significant over mortality for the pre excitation subjects This finding confirms the general concensus of a good prognosis in pre excitation As far as is known only one died during an attack of tachycardia a woman aged 32 previously reported by Öhnell (34)

Thirty one of the 33 subjects still alive in 1965 could be re examined They were between 29 and 84 years of age 19 were males and 12 females The examination included a cardiac history and physical examination an electrocardiogram and a chest X ray

Eight (26 %) of the 31 fulfilled Wolff Parkinson White s or Öhnell s criteria and 15 (48 %) the criteria discussed above In *fourteen of the 16 cases without pre excita*tion electrocardiograms at re examination attempts were made to provoke delta waves by prolonged maximal inspiration and expiration and carotid sinus pressure but *this did not result in* a *diagnosis of pre* excitation in any case Unsuccessful attempts were made in 4 cases to abolish the normalization by bicycle work

The attacks of tachycardia were reported by 17 (55 %) *to have become less frequent* with time and by 5 (16 %) more frequent Sixteen (52 %) said that they had had no attacks during the previous year while four (13 %) had had attacks as often as once or twice a week The patients with and without attacks of tachycardia during the last year did not differ significantly with regard to the frequency of normalized electrocardiograms

Effort angina without relation to attacks of tachycardia occurred in 7 patients (23%) which is a probable over representation compared with the general population ($P < 0.05$)

Probable cardiac insufficiency was diagnosed in one patient (3 %) on the basis of effort dyspnea and cardiac enlargement One

patient had this diagnosis on the death certificate

Two patients had pathological cardiological findings at auscultation One of them was thought to have a slight mitral insufficiency or a small ventricular septal defect (3 %) and the other a non obstructive cardiomyopathy Of those autopsied one had a mitral valvular disease and one a non obstructive cardiomyopathy

In the literature no follow up study was found with which these results could be compared

Studies on heredity

Öhnell (33) described two families in which several brothers and sisters had possible or probable pre excitation electrocardiograms and attacks of tachycardia and it was in one of these that Öhnell (34) at autopsy found a subepicardial muscle bundle which connected the left atrium with the left ventricle dorsally Now 25 years later, there are 189 members alive in these two families, distributed over 4 generations, and, of these, 152 could be examined with reference to pre excitation electrocardiograms and paroxysmal tachycardia At this examination two subjects showed a pre excitation electrocardiogram as did 4 others at the original examination, altogether 6 pre excitation cases by the criteria used here Among these there were siblings and cousins but no parent child combination As pre excitation can occur intermittently, a normal electrocardiogram does not exclude the possibility of the subject being affected Therefore, the question of the genetic basis of pre excitation in these families cannot be settled on the basis of the findings at these two examinations (figs 8 and 9) This result is in accordance with that of Warner and McKusick (54) who found no additional W P W subjects when examining 80 members of 14 W P W families Paroxysmal tachycardia is probably inherited as an autosomal dominant in the two pre excitation families studied here

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APPENDIX

Follow up results of 75 pre excitation cases

Ohnell Birch no	Sex	Age		History				Physical findings	Heart volume ml/m BSA	ECG					
		at death	at follow up 1965	Attacks of tachycardia last year	Cardiac insufficiency	Angina pectoris	Medication			PR csec	Delta wave			QRS csec	Genuine normalization
											lead	csec	slope mV/sec		
Paroxysmal tachycardia at original examination															
O 2	M		47	100	—	—	—	—	550	11	CR_4	1	6	11	
3	F	43													
5	Not found														
6	M		50	25	PND RD	EA	—	LE	430	11	CR_4	1	7	13	
7	F	62													
9	F		51	1	PND	EA	—	—	350	11	II	4	2	10	GN
10	M		73	0	—	EA	—	—	350	17	—	—			
11	F	32													
13	M		36	0	—	—	—	—	100	9	II	1	7	11	
14	F		65	20	—	NA	Q	—	300	11	I	5	5	11	
15	F		51	Not examined											
16	M		63	0	(ED)	EA	—	—	170	20	—	—			
18	M	40													
19	M		56	0	—	—	—	—	390	13	CR_4	1	6	13	
20	F	57													
23	M	21													
26	F	70													
27	M		47	Not examined											
28	M	31													
30	M	27													
31	M	61													
32	F		46	100	—	—	—	—	420	10	I	1	15	11	
33	M		45	0	—	—	—	HI PSM apex 1/6 220/100	480	15	—	—			
34	F	62													
36	M		48	10	—	—	—	—	310	19	—	—			
37	F		57	0	—	—	—	—	460	9	V_4	7	19	12	
38	M		29	0	—	—	—	—	360	11	—	—			
39	Not found														
40	F		57	0	—	—	D	—	370	12	II	1	2	14	
41	M		57	2	—		Q	—	510	22	I	7	6	13	
42	M		51	20	CD PND	FA	D Q	—	315	9	V_4	5	2	11	
44	M		42	10	—	—	—	—	520	13	V_2	4	5	15	
45	M	47													
47	M	80													
48	M	64													
50	M		50	0	—	—	—	—	360	12	CR_2	5	9	13	
51	M	66													
52	Not found														
53	F		61	2	—	—	—	C	420	15	—	—			

51	M		49	0	ED PND RD	—	D Q	—	410	10	I	6	11	12	
55	F		84	0	—	—	—	RD 220/100	410	15	—	—			
56	F		83	0	—	—	D	—	525	16	—	—			
57	M	61													
58	M		43	0	—	—	—	—	350	17	—	—			
60	F		49	0	—	—	—	?	<450	13	—	—			
65	F		51	10	PND	EA	—	—	400	14	—	—			
66	M		65	0	—	EA	D	—	470	16	—	—			
67	M		49	10	—	—	Q	—	350	16	—	—			
68	F		59	1	RD	—	D	ED 170/100	370	16	—	—			
69	M		73	0	(ED)	—	D	(RD) (C)	360	14	—	—			
70	M	55													
B 1	Not found														
2	F		51	100	ED	—	D Q	4 HFESM I_3sin 3/6	480	12	I	7	10	13	
3	M		64	100	—	—	—	—	560	19	—	—			
No paroxysmal tachycardia at original examination															
Ö 1	F		64		—	EA	D	HFESM I_3sin 3/6	455	13	II	4	12	12	
4	M	61													
8	F		36	Not examined											
12	M		75		—	—	D	—	400	14	V_4	5	4	13	
17	F		47	100	—	—	—	—	330	12	II	5	8	13	
21	M		43		—	—	—	—	420	12	I	6	8	12	
22	F		45	1	—	—	—	—	<450	15	—	—			
24	Not found														
25	M	62													
29	Not found														
35	M		49		—	—	—	—	380	17	—	—			
43	M		43	0	—	—	—	—	320	13	CR_5	3	6	12	
46	F		49	10	—	—	—	MFESM I_4 sin 3/6	390	14	—	—			
49	F		64	1	—	—	—	?	<450	12	II	8	3	13	GN
59	M	72													
61	M		67		—	EA	—	Not examined							
62	M		56		—	—	—	—	390	16	—	—			
63	M	71													
64	M	64													
B 4	F	36													
5	M	48													

ED = Effort dyspnea
PND = Paroxysmal nocturnal dyspnea
RD = Dyspnea at rest
EA = Effort angina
NA = Nocturnal angina
D = Digitalis
Q = Quinidine
( ) = Co existing chronic respiratory disease

C = Lip cyanosis
LE = Leg edema
4 = 4th heart sound
HFESM = Early systolic murmur of high frequency maximum point degree
MFESM = Early systolic murmur of medium frequency maximum point degree
HFPSM = *Pansystolic murmur of high frequency*, maximum point degree
GN = Intermittent genuine normalization

Appendix I

Paroxysmal tachycardia cases with delta waves

ECG	Sex	Age	Delta wave				
no			P R *csec*	lead	csec	slope *mV/sec*	QRS *csec*
T1	M	65	17	II	4	3	8
T2	M	54	17	CR_5	4	15	10
T3	M	53	13	II	5	5	11
T4	M	39	17	II	3	11	8
T5	F	48	15	II	3	11	10
T6	M	48	17	II	3	13	10

Appendix II

Pre excitation cases among 2300 health survey electrocardiograms

Sex	Age	P R csec	Delta wave csec	QRS csec	ST J depression mm	Paroxysmal tachycardia
M	39	10	4	11	0	+
F	48	11	6	12	—	+
M	52	17	3	10	—	—
M	60	14	3	11	—	—

Appendix III

Pre excitation cases among 5000 hospital electrocardiograms

Sex	Age	P R csec	Delta wave csec	QRS csec	ST J depression mm	Paroxysmal tachycardia
F	24	10	5	13	1	+
M	38	10	7	15	—	+
M	62	10	7	14	—	—
F	35	10	5	12	2	+
M	35	9	5	11	2	+
M	58	12	6	14	—	+
M	25	11	6	13	0	—
F	24	12	5	10	1	—
M	50	11	6	15	—	+
M	52	12	5	10	—	+
M	58	17	3	8	—	—
M	53	15	6	11	—	+
F	78	12	4	11	—	—
M	63	17	3	9	—	—
M	33	13	3	10	0	+
F	77	17	3	10	—	?
M	67	17	4	14	—	—
M	52	12	3	11	—	—
M	52	13	3	9	—	+

Appendix IV

Causes of death in cases without paroxysmal tachycardia at the beginning of the observation period

Case no	Sex	Age at death	Diagnosis on the death certificate	Autopsy
Ö 4	M	61	Organic heart disease	
5	M	62	Hypertensive heart disea e	
59	M	72	Cerebral hemorrhage	
63	M	71	Carcinoma of the colon	
61	M	64	Multiple myeloma	
B 4	F	36	Rupture of the liver	
5	M	48	Aortic valvular disease	+

Appendix V

ACTA MEDICA SCANDINAVICA



From Medical Department VIII Ullevål Hospital Oslo
(Head Professor Anton Jervell) and Johan Throne Holst's Institute for Nutrition Research,
University of Oslo Norway (Head Professor Ragnar Nicolaysen)

The Effect of Plasma Cholesterol Lowering Diet in Male Survivors of Myocardial Infarction

A controlled clinical trial

BY

PAUL LEREN, M D

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TO

Anton Jervell

CONTENTS

INTRODUCTION

Epidemiological studies have demonstrated an increased incidence of coronary heart disease (CHD) in several geographic areas and populations particularly in males

Malmros (1949, 1950) focussed attention on the correlation between CHD and the changes in dietary in take of cholesterol and fat in the Scandinavian countries

Ström & Jensen (1951) showed that the strict diet restriction in Norway during the last war coincided with a marked fall in mortality from diseases of the circulatory system, which before the war had been rising The diet change was chiefly a reduced supply of calories, mainly from foods containing fat, including those rich in cholesterol

Many factors seem to be related to CHD and its causes and pathogenesis are complex. The plasma cholesterol level is one of the most warmly debated factors Prospective studies demonstrate that the risk of experiencing a clinical episode of CHD is a function of the plasma cholesterol level

In the Framingham study (Dawber et al 1962) the incidence of CHD in men aged 30-49 with a serum cholesterol level of 260 mg per 100 ml or more was more than five times greater than in men in the same age group with a serum cholesterol value below 200 mg per 100 ml.

The long term study at the Western Electric Company in Chicago (Paul et al. 1963) demonstrated a definite association between the serum cholesterol level and the development of CHD

In the Peoples Gas Company study, also in Chicago (Stamler et al 1966), an approximately linear increase in the incidence of new clinical CHD with the serum cholesterol level was demonstrated.

In Norway, Jervell et al. (1965) demonstrated that the cardiovascular mortality in three geographic areas differed in relation to the serum cholesterol level

In Oslo, a three-year follow-up study of 6 886 men (Westlund & Nicolaysen 1966) showed a marked association between morbidity and mortality from CHD and the serum cholesterol level

In Norway, particularly in Oslo,

Chapter 1

EARLIER STUDIES

A Previous controlled trials with plasma cholesterol lowering regimen

Drug trials

1 Oliver & Boyd (1961) divided 100 male survivors of myocardial infarction into 2 random groups, giving estrogen to the experimental group and placebo to the controls A significant fall in blood lipids occurred in the experimental group However, a five-year follow up revealed no difference in mortality or morbidity, 18 in both groups having had reinfarction

2 Marmorston et al (1960, 1962) found that the survival rate of men who had recovered from a myocardial infarction was significantly higher in those treated with Premarin for 75 days or more than in the controls However they failed to find any clinical effect of two other estrogen preparations

3 Stamler et al (1963) randomized 275 male survivors from myocardial infarction below the age of 50 The experimental group received estrogen the controls placebo The observation period was 57 2 months in the controls and 58 8 in the experimental group The observed mortality was 48 2 per cent lower in the treated group than in the controls

Dietary trials

1 Lyon et al (1956) recommended low fat — low cholesterol diet to 280 survivors from myocardial infarction. The allocation of the patients into a diet and a control group was made after a one-year follow up according to the answers of a questionnaire sent to the patients or to the relatives of the deceased. According to the degree of adherence to the previously recommended diet, the material was divided into 155 dieters and 125 controls After a mean observation period of 3 8 years there were 15 reinfarctions, 4 of which were fatal, in the diet group, against 51, including 13 fatal cases, in the control group, after a mean follow up period of 4 2 years

2 Nelson (1956) prescribed low fat diet for 175 patients with CHD or cerebrovascular disease, and followed them for 36-72 months In 26 the blood lipid level did not change satisfactorily, and 39 adhered to the diet

an alarming increase in the incidence of CHD occurred in the post-war years (1945-61) Statistics from Oslo were published by Westlund & Hougen (1956, 1961) and by Westlund (1965) Male Oslo residents, aged 55-64, discharged from five medical departments of internal medicine with a first diagnosis of myocardial infarction, increased from 9 0 per 10,000 population in 1945 to 64 9 in 1961

In this period, mainly because of the disagreement on the relation between dietary fat, plasma cholesterol level, and CHD, a controlled clinical trial was planned, and the actual experiment was started in Oslo in November 1958

At that time a couple of trials had already appeared, and in the course of the present trial some more have been published. A short review of these is therefore warranted

as to the effectiveness of the experimental diet, which is calculated to contain about 40 per cent polyunsaturated fat and 365 mg cholesterol as compared to 12 per cent and 720 mg respectively in the control diet.

Of great interest was the observed change in lipid fatty acids in the serum and in adipose tissue, the linoleic acid content being increased in the dieters In the atheromata of subjects who died during the trial, an increased linoleic acid content of the various lipid fractions containing fatty acids was also observed.

7 Koranyi (1963) reports a Hungarian dietary trial in which 183 post myocardial infarction patients were divided into four diet groups No data are given for the comparability of the groups In group I, fat was restricted to 35-40 g daily, all qualities of fat being allowed. In group II, 50 g sunflower oil was recommended, and saturated fat restricted. In group III, dietary fat was set at 50 g, two-thirds of which was saturated and one-third edible oils Group IV served as controls, and were given no dietary restrictions

In the three groups with fat restrictions, the pathologically elevated serum lipids were said to be permanently normalized. However, no figures were given A report of the results has been given for group I (low fat), in which the 3 years' mortality was 8 6 per cent as compared with 19 7 in the controls

8 The Anti-Coronary Club study (Jolliffe et al. 1963 Christakis et al. 1966) began in 1957 with an investigation of the feasibility of maintaining a large group of male New Yorkers on a plasma cholesterol lowering diet, which was designed so that approximately equal quantities of saturated, polyunsaturated, and monounsaturated fat would supply 30 32 per cent of the total caloric intake

In 1960 a control group was recruited from patients of three New York cancer detection clinics After one year on the study diet the average serum cholesterol level had fallen from 260 to 228 mg per 100 ml, and remained approximately at that level In the control group the initial level averaged 250 mg per 100 ml, and after four years 252

By 1964, 8 new coronary events had occurred in 814 experimental subjects The 463 men in the control group had had 12 new coronary events The difference between the groups is statistically significant.

9 In London a controlled trial ran from 1957 to 1963 (London Hospitals Research Committee 1965) Two hundred and sixty four men under 65 years who had recently recovered from a first myocardial infarction were placed at random on either a low fat diet (40 g daily) or on a normal diet. No attempts were made to alter the nature of the fat consumed. The mean fat intake calculated from dietetic weighings was 45 g daily in the experimental group, and 110-113 g in the controls. The daily caloric intake in the diet group was 1900-2000, as compared with about 2400 in the controls The body weight re-

for less than 12 months These 65 patients were designated as controls After a mean observation time of about 50 months the mortality in the diet group was 10 per cent, against 31 in the controls

3 Morrison (1955, 1960) started a controlled trial in 1946 One hundred patients aged 32-70 having recovered from a myocardial infarction were taken in alternate order, one patient being placed on a low fat, low cholesterol diet, and the next on the usual preinfarction diet. There were 42 men and 8 women in the diet group, mean age 60 years In the control group there were 43 men and 7 women, mean age 62 The experimental group had for 3 years a daily intake of 25 g fat and 50-70 mg cholesterol The daily dietary fat and cholesterol in the controls were judged to be 80-160 g and 200-1800 mg respectively

After 3 years' observation the serum cholesterol had on an average been reduced from 312 to 220 mg per 100 ml in the diet group After a 12-year follow-up all patients in the control group against 31 in the diet group had died, mostly from recurrent forms of atherosclerosis

4 Turpeinen et al (1960) have given a preliminary report on a controlled dietary trial in Finland The ordinary house diet of a mental hospital was changed by replacing whole milk by an emulsion of soy bean oil in skim milk, and by replacing butter and margarine by a special brand of margarine more unsaturated than ordinary margarine After the diet change the mean serum cholesterol was reduced Another mental hospital has been designated to act as control Results of the trial, which was started in 1958, are not yet available

5 In Copenhagen, From Hansen et al (1962) treated elderly institutionalized people The diet group consisted of 133 men, the control group of 132 The mean age was 78 years (65-90), and the duration of the trial was 4 7 months Both groups had the same number of patients with cancer and diabetes Further data of the comparability of the groups are not given In the experimental group butter and margarine were substituted by corn oil and soy bean oil, the total intake of which was approximately 40 g per day Serum cholesterol determinations were regularly performed in 28 dieters After 2-3 months, serum cholesterol had fallen from 184 to 161 mg per 100 ml

In the diet group 2 thromboembolic episodes occurred, against 14 in the controls The statistical analysis does not include thromboembolism in treated patients and controls arising within the first 4 weeks Thus 78 patients in the treated group and 89 in the controls were excluded.

6 In 1959, Dayton et al (1962, 1965) started a controlled clinical trial with elderly institutionalized men, randomly assigned to an experimental and a control group The study is carried out on a double blind basis

The authors state that the data on morbidity and mortality are still too meager to permit valid conclusions

12 Bierenbaum et al (1965) have given a preliminary report of a controlled dietary trial One hundred male patients with documented myocardial infarction and under dietetic management with a 30 calorie per cent fat diet, relatively high in polyunsaturated fatty acids, were matched with an identical nondietary-treated coronary group, and followed for five years

The mean serum cholesterol in the diet group changed from 259 mg per 100 ml on admission to 235 at the time of evaluation. The nondiet group had a 1 6 times higher infarction rate and a 2 3 times higher mortality rate than the study group Both differences were found to be statistically significant.

It may be concluded that the abovementioned estrogen and dietary trials have yielded conflicting results The dietary trials also indicate the great difficulties associated with the selection of an adequate material and the establishment of comparable groups and with the management of such trials

B Pilot study

In order to evaluate the plasma cholesterol lowering effect of the experimental diet, and to obtain practical experience, a pilot study was started in 1957

The main principle of the diet was that it should be low in saturated fat and in dietary cholesterol and rich in polyunsaturated fat.

Such a diet was given to 50 patients during their stay in the hospital for coronary heart disease Serum cholesterol was determined at close intervals in 32 patients after discharge for 8-38 weeks, on an average 22 weeks The dietician who was to participitate in the planned controlled trial instructed the patients during the hospital stay, and also visited them in their homes after discharge

The mean cholesterol reduction obtained in this pilot study was 26 per cent.

Useful experience was gathered It was learned that it could be very difficult to induce a person to change his diet habits even just after having survived a myocardial infarction. Especially important was the experience that continous instruction and supervision were essential to prevent the patients from returning to their previous food habits It became clear that the planned controlled trial had to be based on a vigorous and enduring follow-up of the dieters

mained constant throughout the trial, except for the initial 6 months

Twelve patients admitted to the trial were later rejected because anticoagulant therapy was supposed to be necessary, or because they did not cooperate, or because they relapsed before starting the diet

Serum cholesterol was regularly tested in 31 dieters and 41 controls on admission, at 6, 12, and 24 months of observation, the concentrations (mg per 100 ml) being 263-224-229 and 223 in the dieters, and 266-253-243 and 239 in the controls

The patients were followed-up from 2½ to 6 years, the mean observation time being 3 04 years for the low fat group and 3 05 for the controls

After 4 years the relapse rate was 41 per cent in the low fat group and 39 in the control group It was concluded that in men under the age of 65 who have survived a first myocardial infarction, a low fat diet does not improve the prognosis

10 Another English trial was performed by Rose et al (1965) Eighty patients of both sexes with ischemic heart disease were allocated randomly to three treatment groups, a control group of 26 patients, a second group of 26 given supplements of 50-60 g olive oil daily, and a third group of 28 given 50-60 g corn oil daily In both the groups given oils animal fat was restricted

The serum cholesterol level was significantly reduced in the corn oil group, while the mean values for the controls and the olive oil patients showed no significant change

After a follow-up of 2 years, one patient in the control group, two in the olive-oil group, and three in the corn-oil group had died suddenly Definite myocardial infarction occurred in three controls (no fatalities), in five olive-oil patients (one of which was fatal), and in five corn-oil patients (two of which were fatal)

The authors concluded that corn oil cannot be recommended in treatment of ischemic heart disease, being probably not beneficial and possibly harmful

11 Hood et al (1965) report a Swedish trial From a material of 458 cases of essential hypercholesterolemia observed for 15-17 years, a group of 112 highly selected and actively treated patients was matched with a group that was either untreated, or submitted to short-term attempts at a strict dietetic regimen or medicamental treatment, or who had such minor dietary changes as avoidance of excess of butter and cream The diet of the treated group was a low fat, low cholesterol diet with the addition of oils high in polyunsaturated fatty acids

In the female dieters of this material the decrease of serum cholesterol was somewhat greater than in the matched controls In the males, however, serum cholesterol was in fact less reduced in the treated than in the matched controls

In the treated group 13 died as compared with 21 in the matched controls There was no definite difference between the groups in the total rate of myocardial infarction

12 Bierenbaum et al. (1965) have given a preliminary report of a controlled dietary trial. One hundred male patients with documented myocardial infarction and under dietetic management with a 30 calorie per cent fat diet, relatively high in polyunsaturated fatty acids, were matched with an identical nondietary-treated coronary group, and followed for five years

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PRESENT STUDY

When the present controlled trial was planned it was felt that the ideal approach would be long-term controlled dietary trials with large randomized groups of young people Realizing that such a program could not be put into practice, a controlled clinical trial with patients who had suffered myocardial infarction was planned

However, the use of such a material involves certain drawbacks A negative result might be interpreted in two ways The treatment might be judged generally ineffective, or to be ineffective because the trial was undertaken in subjects with advanced atherosclerosis in whom other factors might have assumed greater prognostic significance than an elevated plasma cholesterol level

Nevertheless, correctly planned and conducted, the trial, even if the results proved to be negative, would give answers to the important practical question whether coronary patients, in addition to their disease, should also be subjected to the strict restrictions entailed in a cholesterol lowering regimen

A positive result, however, would be of importance, not only for the treatment of coronary patients, but also for the approach to primary prevention of coronary heart disease

Chapter 2

MATERIAL AND METHODS

Material

The patients for the present study were taken from the material assembled by the Life Insurance Companies Institute for Medical Statistics at the Oslo City Hospitals The methods used in assembling this material have been described previously (Westlund & Hougen 1961, Westlund 1965) The Institute registered all Oslo male residents aged 30 64 years discharged with the diagnosis of myocardial infarction from 13 medical departments in Oslo

During the three-year period 1956-58 681 patients resident in Oslo and discharged alive were registered. This number does not include

a) patients who before 1956 had been discharged from the departments in question with the diagnosis of myocardial infarction, a total of 198 patients
b) patients admitted to the hospital for other disease but with a history of myocardial infarction, or patients with postinfarction heart decompensation, a total of 54 patients
c) patients discharged with a diagnosis of infarctus cordis imminens, a total of 4 patients

Of the 681 patients, 458 were assigned to the trial, the remaining 223 being excluded for certain pre-established reasons

Table 1 presents the composition of the material and the reasons for exclusion before randomization.

The 458 patients were allocated to the diet and the control group by the Institute, using tables of random numbers Identical letters were sent to both groups, summoning the patients to a medical examination of their previous heart disease

During the periods November 1958 to March 1959, and January to February 1960 in groups of 10-15, 291 and 123 patients respectively were registered for the trial The dieters attended on Tuesdays and the controls on Thursdays or Fridays Equal numbers from each group were called in. The start of the observation time for each batch of controls was from the day of registration of the corresponding dieters, viz. 2-3 days earlier

Twenty-one of the patients drawn

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Nevertheless, correctly planned and conducted, the trial, even if the results proved to be negative, would give answers to the important practical question whether coronary patients, in addition to their disease, should also be subjected to the strict restrictions entailed in a cholesterol-lowering regimen.

A positive result, however, would be of importance, not only for the treatment of coronary patients, but also for the approach to primary prevention of coronary heart disease.

Methods

Examination at the start of the trial
At the start of the trial Professor Jersell kindly assisted me in the clinical examination of the patients About one-half of them were examined by him and one-half by me. During the trial all patients were examined by me only

A medical history was taken. In case of re-admission, the medical records have been reviewed. The examination concerned symptoms of angina pectoris on effort or at rest, symptoms and signs of heart decompensation in the way of dyspnea at rest or pitting edema. Symptoms of peripheral arterial insufficiency in the form of intermittent claudication, in the presence of which pulse palpation of the femoral popliteal and the foot arteries was undertaken. Auscultation of the heart. Blood pressure in the recumbent position after 5 minutes rest. Two readings were taken the lower being registered to the nearest five mm Hg A 12 lead electrocardiogram, including the standard leads I, II, and III, and the unipolar leads aVR, aVL, aVF and V_{1-6} Height and weight. At weighing, the patients took off their shoes, jacket, and vest, and emptied their pockets. The weight of the remaining clothing was estimated at 1,5 kg, which was subtracted from the reading Blood sample for serum cholesterol determination by puncture of a cubital vein with the patient in the sitting position.

Medical treatment was registered. The patients were questioned about their food habits, use of tobacco, and consumption of alcohol, previous to the start of the trial.

When whole milk was not used, and with some moderate restrictions to avoid weight increase, the diet was still considered to be a conventional one. Attempts to reduce dietary fat, but with retention of butter or margarine, and no additional polyunsaturated fat were regarded as moderate measures towards a cholesterol lowering diet. The omission of butter and margarine, the reduction of animal fat, and consumption of additional vegetable oils were considered as a regular cholesterol lowering diet.

With regard to tobacco habits the patients were divided into four groups 1) nonsmokers, 2) those smoking < 10 cigarettes per day or < 50 g tobacco per week in the form of pipe tobacco or self-rolled cigarettes (light smokers), 3) those smoking 10-20 cigarettes per day or 50-100 g of tobacco per week (moderate smokers), and 4) those smoking > 20 cigarettes or > 100 g tobacco (heavy smokers) Because of the frequent combination of self rolled cigarettes and a pipe, it was not possible to consider cigarette smokers alone.

With regard to alcohol they were grouped in three classes 1) no consumption, or only on special occasions, 2) moderate but fairly constant, and 3) considerable consumption with misuse at times

Continous contact with the patients indicated that the information on tobacco was reliable, but not always that about alcohol. In some instances patients then had to be regrouped.

All the data were transferred to special filing cards.

At the start of the trial the patients were told they would be summoned for control examinations at regular intervals for some years, usually twice a year, and that the examination would be free of cost. They were told always to apply to me for help and advice in case of problems concerning their heart disease Other complaints should be referred to their regular doctors

The regular doctors of the patients

Table 1 *Composition of the material and reasons for exclusion*

	Year of discharge			
	1956	1957	1958	Total
Infarction patients registered	195	237	249	681
Diet group	57	88	84	229
Control group	69	71	89	229
	126	159	173	458
Excluded	69	78	76	223
Dead after discharge but before allocation	33	30	14	77
Previously treated in hospital for myocardial infarction	0	0	2	2
Discharged from Rikshospitalet *	7	6	14	27
Moved out of Oslo before allocation	2	2	3	7
Living in lodging houses or without permanent residence	2	2	1	5
Known to be on cholesterol lowering diet before allocation	1	9	11	21
Diagnosis of infarction alternative to non coronary heart disease	2	0	3	5
Cases discovered after start of trial	1	1	0	2
Patients who, in addition to the infarction diagnosis, had the following conditions diagnosed at discharge, or at later hospitalizations				
Diabetes	6	8	7	21
Cerebrovascular disease	2	4	3	9
Syphilis	1	1	2	4
Valvular heart disease	2	1	1	4
Kidney disease	0	3	3	6
Chronic lung disease	4	6	3	13
Chronic rheumatic disease	2	0	0	2
Cancer, polycythemia	2	0	3	5
Progressive muscular dystrophy	0	0	1	1
Psychosis, alcoholism, mental depression	1	4	4	9
Heart decompensation, degree IV	1	1	1	3
	69	78	76	223

* These patients were excluded because they were frequently engaged in therapeutical trials

for the diet group and 23 of the control group did not reply to the letters, and in 2 patients allocated to the diet group the diagnosis of cancer proved to have been made before the allocation These patients were excluded from the trial *

Thus, the present trial was conducted with 412 patients, 206 in the diet group and 206 in the control group

* The total number of deaths in the patients excluded was 7 in the diet group and 10 in the control group in the course of 5 years.

Methods

Examination at the start of the trial

At the start of the trial Professor Jervell kindly assisted me in the clinical examination of the patients About one-half of them were examined by him and one-half by me. During the trial all patients were examined by me only

A medical history was taken. In case of re-admission, the medical records have been reviewed. The examination concerned symptoms of angina pectoris on effort or at rest, symptoms and signs of heart decompensation in the way of dyspnea at rest, or pitting edema. Symptoms of peripheral arterial insufficiency in the form of intermittent claudication, in the presence of which pulse palpation of the femoral popliteal, and the foot arteries was undertaken. Auscultation of the heart. Blood pressure in the recumbent position after 5 minutes rest. Two readings were taken, the lower being registered to the nearest five mm Hg A 12 lead electrocardiogram, including the standard leads I, II, and III, and the unipolar leads aVR, aVL aVF, and V_{1-6} Height and weight. At weighing the patients took off their shoes jacket, and vest, and emptied their pockets. The weight of the remaining clothing was estimated at 1 5 kg which was subtracted from the reading Blood sample for serum cholesterol determination by puncture of a cubital vein with the patient in the sitting position.

Medical treatment was registered. The patients were questioned about their food habits, use of tobacco, and consumption of alcohol, previous to the start of the trial.

When whole milk was not used, and with some moderate restrictions to avoid weight increase the diet was still considered to be a conventional one. Attempts to reduce dietary fat, but with retention of butter or margarine, and no additional polyunsaturated fat were regarded as moderate measures towards a cholesterol lowering diet. The omission of butter and margarine, the reduction of animal fat, and consumption of additional vegetable oils were considered as a regular cholesterol lowering diet.

With regard to tobacco habits the patients were divided into four groups 1) nonsmokers, 2) those smoking < 10 cigarettes per day or < 50 g tobacco per week in the form of pipe tobacco or self-rolled cigarettes (light smokers), 3) those smoking 10-20 cigarettes per day or 50-100 g of tobacco per week (moderate smokers), and 4) those smoking > 20 cigarettes or > 100 g tobacco (heavy smokers) Because of the frequent combination of self rolled cigarettes and a pipe, it was not possible to consider cigarette smokers alone.

With regard to alcohol they were grouped in three classes 1) no consumption, or only on special occasions 2) moderate but fairly constant, and 3) considerable consumption with misuse at times.

Continous contact with the patients indicated that the information on tobacco was reliable, but not always that about alcohol. In some instances patients then had to be regrouped

All the data were transferred to special filing cards.

At the start of the trial the patients were told they would be summoned for control examinations at regular intervals for some years, usually twice a year, and that the examination would be free of cost. They were told always to apply to me for help and advice in case of problems concerning their heart disease Other complaints should be referred to their regular doctors

The regular doctors of the patients

were all contacted in person about the trial, and their permission to use their patients in the trial was asked and given The doctors were informed to which group their patients belonged, and they were asked not to suggest any change of diet The hospitals in which the patients had stayed during the primary infarction were also informed about the trial All my colleagues were very responsive and ready to contribute to the accomplishment of the trial

At the start of the study it was decided that each patient should remain in the trial for exactly 5 years

The cholesterol method

Total serum cholesterol was analyzed with the method of Hauge & Nicolaysen (1958) The cholesterol method has been thoroughly checked by the Sperry Webb method during the last years, and the two have given the same results Cholesterol analyses are under rigid daily control, reference serum standard being used The cholesterol standard used is chromatographically pure (Grav & Nicolaysen, personal communication, 1966)

Comparability of the test groups

The results of the testing of the comparability of the groups at the start of the trial are presented in Table 2

Table 2 *Comparability of the groups*

Diet group	206 patients
Control group	206 »
	412 patients

I. *Characteristics of groups in hospital for primary infarction*

A. Hospital department

Department	Diet	Control
Ullevål, dept. I	3	2
» » VII	51	49
» » VIII	46	56
» » IX	35	29
» » XVB	0	0
» » Krohgstøtten	5	8
Aker med dept. A	41	42
» » » B	10	6
Diakonissehuset, med dept.	3	5
Diakonhjemmet, » »	8	7
Menighetssøsterhjemmet med dept.	4	2
Total	206	206

B Profession

	Diet	Control
Independent business	21	21
» skilled worker	2	2
Senior employees	14	11
Office and shop employees	53	62
Craftsmen	40	32
Foremen	19	27
Laborers and motormen	57	51
Total	206	206

C Shock

	Diet	Control
Definite	7	8
Probable	9	10

D Relative heart volume (ml per m² body surface area)

	Diet	Control
-499 ml	132	153
500-599 »	32	27
600 »	14	8
Not determined	28	18
Total	206	206

E. Long term anticoagulant therapy prescribed at discharge

	Diet	Control
Yes	183	187
No	23	19
Total	206	206

II. *Events during the time between the discharge from the hospital and start of the observation time*

A. Myocardial reinfarction and cerebral stroke

	Diet	Control
One reinfarction	6a	8b
Two or more reinfarctions	0	0
Cerebral stroke	1c	2d

[a] Of these 4 were without further CHD relapses, 1 died suddenly and 1 had a fatal myocardial reinfarction.

[b] Of these 6 were without further CHD relapses 1 died suddenly and 1 survived a myocardial reinfarction.

[c] This patient died of pneumonia possibly complicated by a cerebral insult.

[d] Both were without CHD relapses (and further cerebral insults)

B Changes in long term anticoagulant treatment

a) Anticoagulant treatment at discharge

	Diet	Control
Treatment discontinued within 6 months after discharge	6	11
Treatment discontinued within 12 months after discharge	6	12
Treatment discontinued during the time between 12 months after discharge and start of observation time	7	19
Anticoag treatment discontinued during the time between the discharge and start of the observation time	19	42

b) Without long term anticoagulant treatment at discharge

	Diet	Control
Treatment started within 6 months after discharge	0	0
Treatment started within 12 months after discharge	1	1
Treatment started during the time between 12 months after discharge and start of the observation time	0	2
Anticoag treatment started during the time between the discharge and start of the observation time	1	3

III. *Characteristics of groups at the start of the observation time*

A. Age (year of start minus year of birth)

	Diet	Control
30-34	0	3
35-39	0	4
40-44	12	9
45-49	23	18
50-54	43	36
55-59	56	53
60-64	49	56
65-(67)	23	27
Total	206	206
Mean age	56 2	56.3

B Work

	Diet	Control
In work (including persons with temporary sick certificates for intercurrent diseases)	170	169
Retired old age pensioners	4	6
Unable to work (still on the sick list or on disability pension)	32	31
Total	206	206

C Marital status

	Diet	Control
Married	182	191
Single	24	15
Total	206	206

D Height and weight (Natvig 1956)

		Diet	Control
Underweight	> 20 %	0	1
»	16—20 %	6	4
»	11—15 %	7	5
Normal weight	(± 10 %)	124	113
Overweight	11—15 %	19	37
»	16—20 %	16	17
»	21—25 %	22	9
»	26—30 %	5	13
»	31—35 %	3	5
»	36—40 %	2	0
»	> 40 %	2	2
Total		206	206
Mean weight (kg)		73.2	74.3
Mean height (cm)		172 4	173.6

E Food habits (definitions p 15)

	Diet	Control
Conventional food habits	170	168
Moderate cholesterol lowering measures	33	37
Regular cholesterol lowering regimen	3	1
Total	206	206

F Tobacco (definitions p 15)

	Diet	Control
Non smokers	70	76
Light smokers	49	49
Moderate smokers	72	63
Heavy smokers	15	18
Total	206	206

G Alcohol

	Diet	Control
None, or only on special occasions	152	150
Fairly regular but moderate consumption	43	47
Considerable consumption with occasional alcohol problems	11	9
Total	206	206

H. Angina pectoris

	Diet	Control
No angina	75	79
Angina on effort	122	119
Angina at rest	(8)	(5)
Uncertain	9	8
Total	206	206

I. Heart decompensation

	Diet	Control
Pitting edema	4	1
Dyspnea at rest	3	2
Uncertain	1	1

J Intermittent claudication

	Diet	Control
Present	4	9
Uncertain	0	2

K. Xanthoma and xanthelasma

	Diet	Control
Xanthomata	9	6
Xanthelasmata	1	3

L. Blood pressure

		Diet	Control
Systolic B.P	Normotensive (-160)	127	154
	Hypertensive (165-)	79	52
Total		206	206
Diastolic B.P	Normotensive (90)	84	110
	Border values (95 100)	56	59
	Hypertensive (105-)	66	37
Total		206	206
Mean systolic B.P		159 0	153 8
Mean diastolic B P		97 1	93 5

M. Electrocardiographic registration

	Diet	Control
Normal or approximately normal	82	85
Atrial fibrillation or flutter	1	2
Atrioventricular block (I degree)	0	1
Left bundle branch block	1	1
Right bundle branch block	2	7
Pathologic QRS changes other than bundle branch block, and pathologic ST- and T changes (changes of inveterated infarction and hypertrophy)	120	112

Definitions

Normal or approximately normal
An ECG which conventionally would have been described as normal.

N Long term anticoagulant treatment

	Diet	Control
Yes	165	148
No	41	58
Total	206	206

O Serum cholesterol

		Diet	Control
150-174 (mg per 100 ml)		1	1
175-199	»	2	5
200 224	»	12	10
225-249	»	22	26
250-274	»	35	34
275 299	»	35	39
300 324	»	46	35
325-349	»	20	25
350-374	»	17	14
375-399	»	9	6
400 424	»	2	6
425-449	»	1	2
450-474	»	3	1
475-499	»	0	0
500-	»	1	2
Total		206	206
Mean value (mg per 100 ml)		296 1	296.2

A statistical analysis of the Table comparing the groups demonstrates no statistically significant differences on the five per cent level, with the exception of height/weight ($p = 025$), and blood pressure ($p = 007$ for systolic and 004 for diastolic pressure)

In Table 2, part II Ba, discontinuing anticoagulant treatment before the start of the trial, there is also a difference However, of importance is the distribution of anticoagulant treatment at the start of the trial, in which there was no statistical difference between the groups

The readings of the blood pressure were made after the randomization, and by two persons A personal bias, therefore, cannot be excluded The blood pressure values also demonstrate a digit preference No decisive importance can therefore be attached to these minor differences, although they are technically statistically significant.

Therefore, from the testing of the comparability of the groups at the start of the trial, it seems justified to conclude that the groups ran an equal risk of acquiring CHD relapses

Evaluation of the results of the trial

The results of the present trial have been judged by the incidence of myocardial reinfarction, new cases of angina pectoris, and sudden, unexplained death. These events have been called CHD relapses

The reasons for this order of priority of the CHD relapses are the fol-

D Height and weight (Natvig 1956)

		Diet	Control
Underweight	> 20 %	0	1
»	16—20 %	6	4
»	11—15 %	7	5
Normal weight	(± 10 %)	124	113
Overweight	11—15 %	19	37
»	16—20 %	16	17
»	21—25 %	22	9
»	26—30 %	5	13
»	31—35 %	3	5
»	36—40 %	2	0
»	> 40 %	2	2
Total		206	206
Mean weight (kg)		73.2	74 3
Mean height (cm)		*172 4*	*173.5*

E Food habits (definitions p 15)

	Diet	Control
Conventional food habits	170	168
Moderate cholesterol lowering measures	33	37
Regular cholesterol lowering regimen	3	1
Total	206	206

F Tobacco (definitions p 15)

	Diet	Control
Non-smokers	70	76
Light smokers	49	49
Moderate smokers	72	63
Heavy smokers	15	18
Total	206	206

G Alcohol

	Diet	*Control*
None, or only on special occasions	152	150
Fairly regular but moderate consumption	43	47
Considerable consumption with occasional alcohol problems	11	9
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H. Angina pectoris

	Diet	Control
No angina	75	79
Angina on effort	122	119
Angina at rest	*(8)*	*(5)*
Uncertain	9	8
Total	206	206

I. Heart decompensation

	Diet	Control
Pitting edema	4	1
Dyspnea at rest	3	2
Uncertain	1	1

J Intermittent claudication

	Diet	Control
Present	4	9
Uncertain	0	2

K. Xanthoma and xanthelasma

	Diet	Control
Xanthomata	9	6
Xanthelasmata	1	3

L. Blood pressure

		Diet	Control
Systolic B P	Normotensive (-160)	127	154
	Hypertensive (165-)	79	52
Total		*206*	*206*
Diastolic B.P	Normotensive (-90)	84	110
	Border values (95 100)	56	59
	Hypertensive (105-)	66	37
Total		206	206
Mean systolic B.P		159 0	153 8
Mean diastolic B P		97 1	93 5

conclusions of two of the members were not in agreement, the conclusion of the third member was decisive

As a guidance for the diagnosis of myocardial reinfarction a code of diagnostic criteria was established (Table 3)

Table 3 *Diagnostic criteria for myocardial reinfarction*

A. Typical development of new ECG changes characteristic of myocardial infarction

B The development of a Q wave not previously registered in addition to
  1 Typical chest pains
  or to at least two of the following criteria
  2 Typical rise in body temperature
  3 Leucocyte count $\geq$ 10 000 per mm[3]
  4 Increase in the sedimentation rate of 50 per cent or more of the first value but at least up to 15 mm per hour or a definite and gradual fall of an elevated value (Method of Westergren)
  5 Rise in SGOT to $\geq$ 50 units, or $\geq$ 100 per cent increase of the first value, but at least up to 40 units or a $\geq$ 50 *per cent fall from a minimal value* of 30 units (Reitman et al. 1957)
  6 Pulmonary edema, clinically
  7 Pericardial friction murmur

C ECG changes uncertain or absent
  1 Typical chest *pains in* addition to least two of criteria 2 7

Follow up studies during observation time

All examinations were performed in the out patient clinic of medical department VIII, Ullevål Hospital, and solely by me technically assisted only in the recording of the electrocardiograms and in the drawing of blood for serum cholesterol determinations The days of the week were equally divided between the groups The examinations were usually made between 1 and 4 p.m. A few patients were also examined at 8—9 a.m and at 5—7 p m. These early and late examinations were equally frequent in the two groups

The first examination took place 3 months after the start of the trial From then on the patients were controlled twice a year Thus, each surviving patient was examined 11 times in all In the vacation periods from the middle of June to the middle of August and from the middle of December to the middle of January, no examinations were made

The follow up examinations included History of the time elapsed since last examination, with special emphasis on possible hospitalizations and absence from work through sickness Presence of angina pectoris amelioration and deterioration Symptoms and signs of heart decompensation in the form of dyspnea at rest or pitting edema and of peripheral arterial insufficiency Changes in the medicamental therapy, especially with regard to long term anticoagulant therapy Diet examination of the dieters, and cautious questioning of the food habits of the controls Auscultation of the heart, reading of blood pressure, and weight control. Blood samples for cholesterol determination. A 12 lead ECG every second examination, or at each if there was any reason to believe from the history that ECG changes might have occurred.

After each examination the medical records from hospitalizations were provided and possible reinfarctions decided by the diagnostic board.

In Tables 4 and 5 are listed all patients

lowing whereas myocardial reinfarction and new cases of angina pectoris are assumed to be more directly related to the degree of atherosclerosis and thus possibly influenced by dietetic measures, sudden death in subjects who have survived a *myocardial* infarction may not In such *persons*, sudden death is presumably more related to the localization of the atherosclerotic changes than to the degree, the immediate cause of death most often being electrical instability due to ischemia

Therefore, it was *not likely* that dietetic measures, even when antiatherogenic, would influence the incidence of sudden death in the patients of the present study with probably advanced atherosclerosis, who had all survived a myocardial infarction

Definition of CHD relapses

A Myocardial reinfarction

Type I Clinical diagnosis made by a diagnostic board according to preestablished diagnostic criteria

Type II Diagnosis made by autopsy

Type III Fatal episodes with chest pains preceding death No clinical or laboratory diagnosis established Autopsy performed, but showing no definite fresh myocardial infarction or any other cause of death (Case reports p 44)

B Acquired angina *pectoris*

Patients without angina pectoris at the start of the observation time who acquired angina pectoris during the trial

In making the diagnosis of angina *pectoris no* physical or laboratory tests *were used* The decision was made entirely according to the presence of the characteristic symptoms and their relation to effort and rest, and to the typical effect of nitroglycerine

C Sudden death (including unexplained, probably coronary death)

Type I Death witnessed Patients dying instantaneously Autopsy if performed failed to reveal cause of death

Type II Death witnessed Fatal episodes with chest pains preceding death No clinical or laboratory diagnosis established Autopsy *not* performed

Type III Death unwitnessed Patients found dead, having been observed alive shortly (minutes to hours) before No signs of unnatural death Autopsy if performed failed to reveal cause of death

The diagnosis of a new myocardial infarction was left to a diagnostic board consisting of three senior physicians In cases of a possible reinfarction all medical records including the electrocardiographic material of the study were submitted to the members of this board, who, independently of each other, and not knowing whether the actual patient was a dieter or a control, reported in writing if a reinfarction had occurred The decision was made by at least two members of the board If the

In Table 6 data are presented on the 5 patients, 3 in the diet group and 2 in the control group, of whom regular control had to be stopped because of cerebral stroke or mental deterioration. None of these suffered relapses of CHD One patient in each group died of cerebral stroke.

Table 6 *Cerebral stroke Mental deterioration*

Pat. No	Cause	Regular control stopped (months after start)	Institutional care	Alive/dead at end of trial	Cause of death (months after start)
D-65	Stroke	44½	Yes	Alive	
D-104	Mental deterioration	44	Yes	Alive	
D 155	Stroke	9	Yes	Dead	Cerebral stroke (54 months) No autopsy
C-47	Mental deterioration	28	Yes	Alive	
C-111	Stroke	19½	Yes	Dead	Cerebral thrombosis (19½ months) Autopsy *

Autopsy revealed a large cerebral infarct, and a dissecting aortic aneurysm reaching from the arch to the lower abdominal part. However no definite changes of a previous myocardial infarction were found.

Table 7 *Cancer during the observation time*

Pat No	Site	Diagnosis established (months after start obs.)	Treatment	Histology / Autopsy	Alive (end of obs.) Dead (months after start obs.)	Cause of death
D-170	Stomach	7	Gastrectomy	Histology +	Alive	
D 62	Lung	46	None	Autopsy +	Dead (56)	Cancer
D 70	Colon	2	Hemicolectomy	Histology +	Alive	
D-93	Colon	42	Hemicolectomy	Histology +	Alive	
C-93	Rectum	53	Explor laparatomy	Autopsy +	Dead (55)	Cancer
C-99	Stomach	2	Explor laparatomy	Autopsy +	Dead (3)	Cancer
C-139	Prostate	7	Estrogens	Autopsy +	Dead (55)	Sudden death
C-206	Pancreas	8	Explor laparatomy	Histology +	Dead (11)	Cancer

who were not observed the whole 5-year period In addition to those who died of CHD there were 17 in the diet group and 9 in the control group who for various reasons could not be regularly controlled However, all of them were followed up until death or the end of the observation time by means of personal interviews or by contact with their physicians and relatives The medical records from hospital admissions were studied, and in case of possible reinfarction the records were made available for the diagnostic board.

Table 4 *Regular control discontinued Causes and point of time*

Diet Group

	Fatal reinfarction	Sudden death	Fatal pneumonia	Death of unknown cause	Stroke Mental deterioration	Cancer	Reluctance	Total
Start								0
3 months		1				1	3	5
9 »	1	4			1	2	3 + 1*	12
15 »	1	6			1	2	3 + 1*	14
21 »	2	9			1	2	3	17
27 »	3	11		1	1	2	3	21
33 »	5	14		1	1	2	5	28
39 »	5	16	1	1	1	2	5	31
45 »	7	22	1	1	3	3	7	44
51 »	7	23	1	1	3	4	8	47
60 »	10	27	1	1	3	4	8	54

* This patient died of myocardial infarction 16 months and 18 days after the start of observation and is from the 21 month examination listed in the column Fatal reinfarction

Table 5 *Regular control discontinued Causes and point of time*

Control Group

	Fatal reinfarction	Sudden death	Fatal aortic thrombosis	Stroke Mental deterioration	Cancer	Emigration	Reluctance	Total
Start								0
3 months	2				1			3
9 »	5	2			2	1		10
15 »	6	4			2	1		13
21 »	7	4		1	2	1		15
27 »	8	9		1	2 + 1*	1	1	23
33 »	13	14		2	2 + 1*	1	1	34
39 »	14	19		2	2 + 1*	1	1	40
45 »	19	21		2	2 + 1*	1	1	47
51 »	21	21		2	2 + 1*	1	1	49
60 »	23	27	1	2	3	1	1	58

* This patient died suddenly and unexpectedly 54 months and 19 days after start of observation. On the 4th day after uneventful operation for appendicitis being in good condition and having been observed shortly before by the nurse he was found dead in his bed Autopsy showed carcinoma of the prostate with metastasis severe coronary atherosclerosis with old myocardial infarction. No thrombus formation or fresh myocardial infarction For the 60-month examination listed in the column Sudden death

In Table 6 data are presented on the 5 patients, 3 in the diet group and 2 in the control group, of whom regular control had to be stopped because of cerebral stroke or mental deterioration. None of these suffered relapses of CHD One patient in each group died of cerebral stroke.

Table 6 *Cerebral stroke Mental deterioration*

Pat. No	Cause	Regular control stopped (months after start)	Institutional care	Alive/dead at end of trial	Cause of death (months after start)
D-65	Stroke	44½	Yes	Alive	
D 104	Mental deterioration	44	Yes	Alive	
D-155	Stroke	9	Yes	Dead	Cerebral stroke (54 months) No autopsy
C-47	Mental deterioration	28	Yes	Alive	
C-111	Stroke	19½	Yes	Dead	Cerebral thrombosis (19½ months) Autopsy

Autopsy revealed a large cerebral infarct, and a dissecting aortic aneurysm reaching from the arch to the lower abdominal part. However no definite changes of a previous myocardial infarction were found.

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D 62	Lung	46	None	Autopsy +	Dead (56)	Cancer
D 70	Colon	2	Hemicolectomy	Histology +	Alive	
D-93	Colon	42	Hemicolectomy	Histology +	Alive	
C-93	Rectum	53	Explor laparatomy	Autopsy +	Dead (55)	Cancer
C 99	Stomach	2	Explor laparatomy	Autopsy +	Dead (3)	Cancer
C-139	Prostate	7	Estrogens	Autopsy +	Dead (55)	Sudden death
C 206	Pancreas	8	Explor laparatomy	Histology +	Dead (11)	Cancer

In Table 7 data are presented on the patients, 4 in each group, in whom a diagnosis of cancer was made during the trial Regular control examinations were stopped when the diagnosis of cancer was made One in the diet group, and 3 in the control group died of cancer In the diet group 1 cancer patient acquired angina pectoris In the control group 1 suffered sudden, unexpected death after having acquired angina pectoris (case report in Table 5)

In Table 8 data are listed of the patients, 9 in the diet and 1 in the control group, of whom regular control was not performed because of bad cooperation or reluctance. One of the reluctant dieters died of myocardial infarction, and the reluctant control survived a myocardial reinfarction

Table 8 *Reluctance*

Pat No	Last examination (months after start)	Reason	Alive (end of obs) Dead (months after start)
		Diet group	
4	3	Venepuncture	Dead (17 reinfarction)
21	0	Diet	Alive
66	39	Diet	Alive
69	0	Diet	Alive
119	39	Diet (Religious reasons)	Alive
124	27	Diet	Alive
140	0	Diet (Alcoholism)	Alive
172	27	Diet (Religious reasons)	Alive
169	45	Diet	Alive
		Control group	
56	21	Reinfarction	Alive

Case reports of the last 4 patients not regularly controlled

Died of pneumonia (D-198) Incapable of work because of increasing mental deterioration Admitted to a hospital two days before death with symptoms of an infection of the respiratory tract and of cerebral confusion Clinical signs of pneumonia were found. Treatment with penicillin. Death occurred 34 months and 18 days after the start of the trial Autopsy not performed

Died of unknown cause (D-156) Cerebral stroke 18 months after the start of the trial, with transitory pareses. Found dead in his bed at home, where he lived alone, 22 months and 26 days after the start of the trial On coroner's request examined by the forensic medical officer No signs of violent or unnatural death Death was estimated to have occurred 7—8 days before he was found. Autopsy was not performed, and no cause of death could be established

Died of aortic thrombosis (C-61) Thrombarterectomy for thrombotic occlusion of the abdominal aorta Death occurred on the first postoperative day, 54 months and 21 days after the start of the trial Autopsy revealed operated aortic trombosis and an old myocardial infarction

Emigration (C 92) Emigrated to Sweden Interview by letter at the end of the trial revealed no episodes or symptoms of CHD relapses

Statistical methods

The number of persons at risk at the start of the trial is the same for both groups, and all survivors have been followed for exactly five years Therefore, it has not been deemed necessary to compute, for each indi-

vidual patient, the exact period of risk for the various types of relapses Rather, the results have been expressed as rates where the numerator contains the persons who got a reinfarction, acquired angina pectoris or died suddenly, and the denominator all patients present at the start of the trial

Except where otherwise specified, only one CHD relapse is counted for each patient The order of priority reinfarction, acquired angina pectoris, sudden death

The experiences in the various portions of the 5-year observation period have been shown by means of cumulative numbers of patients who have suffered relapses at the end of each year

It should be noted that whereas all patients were at risk of reinfarction or sudden death, a new angina pectoris could arise only in patients who were free of this condition at the start of the trial 'Total CHD relapses' being the sum of patients getting a reinfarction, acquiring angina pectoris, or dying suddenly, has therefore given rise to hybrid rates However, in each comparison the number of patients without angina pectoris at the start has been specified in addition to the total number of patients at risk, so that, if desired, a rate for acquired angina pectoris can be computed on the basis of those effectively at risk only

Tests of significance have been made by means of simple x^2 tests with correction for continuity according to Yates

Chapter 3

THE PLASMA CHOLESTEROL-LOWERING DIET

Numerous papers have been published describing and evaluating the plasma cholesterol depressive effect of various types of fat. It is not proposed to review the literature of these well-established effects

Kinsell et al (1952) and Groen *et al (1952)* first observed the cholesterol-lowering effect of vegetable oils, rich in polyunsaturated fatty acids, especially linoleic acid

In rats the marine polyenoic fatty acids with 5—6 double bonds seem to be more cholesterol-lowering than linoleic acid (Nicolaysen & Ragaard 1961) In man no such distinct difference has been established

Saturated fatty acids increase blood cholesterol, however, the effect seems to be limited to the C 12, C 14, and C 16 saturated fatty acids (Keys et al 1965, Hegsted et al 1965)

Oleic acid seems to have little or no effect on the serum cholesterol level (Malmros & Wigand 1957, Keys et al 1958)

Earlier it was thought that dietary cholesterol had little or no effect on blood cholesterol Lately, numerous observations to the contrary have been reported (Malmros 1965, Connor et al 1965) It has also been shown that the higher the dietary cholesterol, the more polyunsaturates are required to reduce serum cholesterol levels adequately (Brown et al 1965) According to Keys et al (1965) serum cholesterol is increased by the square root of dietary cholesterol, whereas Hegsted et al (1965) find an increase of 6 mg per 100 mg dietary cholesterol

The construction of the experimental diet followed the well-known present-day principles of reduction of blood cholesterol The diet should be low in saturated fats and in cholesterol, and rich in highly unsaturated fats The pilot study had also demonstrated a strong cholesterol-lowering effect of such a diet.

Dealing with persons who had survived a myocardial infarction, it was decided to make the cholesterol-reducing effect of the diet as strong as possible By energetic instruction and training it was still hoped that the greater part of the dieters would adhere closely to the rather strict diet.

To prevent instructions being passed on to the patients in the control group it was decided to use oral

instructions only In more detail, the dietetic instructions were as follows

Meat should be restricted as much as possible, and when used, all visible fat should be removed When boiled meat was eaten, the fat layer following cooling should be removed. Whale beef a not uncommon meat in Norway, and poultry were recommended as substitutes for beef, mutton, and pork

Fish of all types, and all kinds of shell fish were recommended

Whole milk and cream should be completely eliminated with the exception of one deciliter of milk with the Sunday dessert and to use in coffee Skim milk was recommended. *Butter* and *fat cheeses* should likewise disappear from the diet. Cheeses of a low fat content should be substituted for the usual cheeses rich in fat.

One egg with yolk was allowed once a week, and egg yolks occasionally used for baking purposes in the homes were accepted Otherwise, egg whites could be used in cooking and baking

A loaf made with whole milk and some extra margarine was not to be used Otherwise, the common types of bread — wheat and rye — were recommended and the use of brown bread was encouraged. Cereals, such as corn flakes shredded wheat, and puffed rice used with skim milk were recommended for breakfast and as a second course at dinner Porridge made with skim milk or water was to be used. Pure sugar should not be used abundantly

With the exception of coconuts, all *foods of vegetable origin*, such as salads, beans, peas, cabbage, carrots, fruit, and nuts, were recommended.

The average consumption of butter and *margarine* per head of the Norwegian population per day has in the years 1953—63 been about 12 g and 65 g respectively When this study started, a typical Norwegian margarine contained only 2—4 per cent of linoleic acid, and the instructions about elimination of saturated fats had to be *very strong with regard* to margarine, which was entirely restricted.

Lard and *shortenings* were also restricted. Use of *olive oil* was discouraged.

At the start of the trial *soy bean oil* was the only oil rich in linoleic acid readily available in the market. Abundant use of this oil was recommended, and it should be used for all cooking and baking As a substitute for margarine and butter, a mixture of skim milk powder and soy bean oil with some salt and coloring material was used.

The total amount of dietary soy bean oil desirable was set at half a liter per week. If it proved impossible to use so much in the cooking and baking and in the butter substitute the daily consumption of 15—30 g soy bean oil as such, taken as a 'medicine', was recommended.

The art of using soy bean oil had to be taught in great detail, in white and brown sauces, in salad dressings, mixed with lemon or mustard as a melted butter substitute with boiled

Chapter 3

THE PLASMA CHOLESTEROL-LOWERING DIET

Numerous papers have been published describing and evaluating the plasma cholesterol depressive effect of various types of fat. It is not proposed to review the literature of these well established effects

Kinsell et al (1952) and Groen et al (1952) first observed the cholesterol-lowering effect of vegetable oils, rich in polyunsaturated fatty acids, especially linoleic acid.

In rats the marine polyenoic fatty acids with 5—6 double bonds seem to be more cholesterol-lowering than linoleic acid (Nicolaysen & Ragaard 1961) In man no such distinct difference has been established.

Saturated fatty acids increase blood cholesterol, however, the effect seems to be limited to the C 12, C 14, and C 16 saturated fatty acids (Keys et al 1965, Hegsted et al 1965)

Oleic acid seems to have little or no effect on the serum cholesterol level (Malmros & Wigand 1957, Keys et al 1958)

Earlier it was thought that dietary cholesterol had little or no effect on blood cholesterol Lately, numerous observations to the contrary have been reported (Malmros 1965, Connor et al 1965) It has also been shown that the higher the dietary cholesterol, the more polyunsaturates are required to reduce serum cholesterol levels adequately (Brown et al 1965) According to Keys et al (1965) serum cholesterol is increased by the square root of dietary cholesterol, whereas Hegsted et al (1965) find an increase of 6 mg per 100 mg dietary cholesterol

The construction of the experimental diet followed the well-known present-day principles of reduction of blood cholesterol The diet should be low in saturated fats and in cholesterol, and rich in highly unsaturated fats The pilot study had also demonstrated a strong cholesterol-lowering effect of such a diet

Dealing with persons who had survived a myocardial infarction, it was decided to make the cholesterol-reducing effect of the diet as strong as possible By energetic instruction and training it was still hoped that the greater part of the dieters would adhere closely to the rather strict diet.

To prevent instructions being passed on to the patients in the control group it was decided to use oral

Table 9 *Diet adherence*
List of scores (Top score 50)

1 *Margarine - butter*		5 *Meat* (Visible fat removed and minced meat avoided)	
More than 250 g per week	0	No	0
50-250 » » »	3	Rarely	3
0- 50 » » »	8	Yes	6
2 *Whole milk*		6 *Eggs*	
More than 1 liter per week	0	More than 3 eggs per week	0
½ 1 » » »	3	2-3 » » »	3
0-¼ » » »	8	0-1 » » »	5
3 *Cream*		7 *Cakes, pastry*	
Daily	0	Daily	0
Rarely	2	None or rarely	5
None	4		
4 *Cheese*		8 *Soy bean oil*	
More than 60 g per day	0	0- 50 g per week	0
20-60 » » »	2	50 250 » » »	3
0-20 » » »	6	More than 250 » » »	8

mended amount of soy bean oil, and actually it never happened

Scores between 34—41 were defined as 'good' adherence With liberal use of margarine and the omission of soy bean oil, the dieter could not be classified as a 'good' adherer, even when adhering completely with regard to all other food prescriptions

Scores between 25—33 were defined as 'fair' adherence To score at least 25, it was necessary to obtain a medium score for all food groups, with the exception of two groups in which top score was necessary

A score less than 25 gave the dieters the classification of 'bad' adherers, although they might have

Table 10 *Degree of adherence to the prescribed diet according to questionnaires issued 6 times during the observation time (per cent of patients)*

	3 months	15 months	27 months	39 months	51 months	60 months	Mean	Mean cholesterol reduction (*per cent*)
Excellent (score ≥ 42)	59 8	63.8	64.4	65 6	67.1	57.5	62.2	21 6
Good (score 34 41)	21.6	18 9	18.1	17.8	15 0	22.5	22.1	12 4
Fair (score 25 33)	11.3	11.7	11.2	8.3	9 6	10 0	10.3	6.7
Bad (score < 25)	5 8	3 6	4.8	5.5	3.5	5 0	3 9	4 0
Reluctant	1.5	2.0	1.5	2.8	4.8	5 0	1.5	—
	100 0	100 0	100 0	100 0	100 0	100 0	100 0	
Mean score	41.5	41.6	41.3	42 4	42 6	41.3	41 6	17 6

fish, in baking of sweet biscuits without the use of egg yolks, etc Frying in oil was a procedure requiring intensive instruction, and so was the production of the butter substitute

A store of oil was kept all the time at the hospital, so that those needing such assistance could be supplied with oil free of charge This service was requested by about 25 per cent of the dieters

Alcoholic and non-alcoholic beverages were allowed

To ensure that the recommended diet fulfilled the requirements for vitamins, but mainly as a means of contact, all dieters were given, free of cost, a multiple vitamin preparation * of which one tablet was to be taken daily The controls were also given the same preparation

As a means of contact and stimulation, the dieters twice during the trial also received, free of cost, considerable quantities of Norwegian sardines canned in cod liver oil, which proved to be popular as a bread spread

Much emphasis was laid on continous instruction and supervision of the dieters during the trial Thus, the dietician paid visits to the homes of the dieters at regular intervals, and also kept contact with the dieters and their families by letter and telephone The dieters were instructed to call on the dietician or on me when in doubt concerning dietetic questions Further, at each of the clinical and laboratory examinations during the trial, all dieters were examined and reinstructed about the diet.

* Biovit 'Afi' Content of one tablet Vitamin A 5000 I U, vitamin D 500 I U, thiamine 2 mg, riboflavin 3 mg, niacine 20 mg, pyridoxin 2 mg, calcium pantotenate 3 mg, vitamin C 50 mg

Control of adherence to the prescribed diet

By means of the frequent and close contact it was possible to form a well-founded opinion of the degree of adherence In addition, a dietary questionnaire was sent to the dieters 6 times during the trial, viz at 3, 15, 27, 39, 51, and 60 months after the start There was quite good agreement between these ways of diet control The results of the answers to the questionnaires, however, being more fit to quantify, will be reported.

For this purpose the degree of avoidance of each restricted food group and the degree of adherence to the prescribed consumption of soy bean oil were given scores according to a list so constructed as to give a score of 50 as a maximum (Table 9) This score would represent the ideal adherence to the recommended diet. According to the score obtained by each dieter 6 times during the observation period, the dieters were divided into four degrees of adherence

A score ≥ 12 was defined as 'excellent' adherence Theoretically it would be possible to be classified as 'excellent' even when using butter or margarine, or omitting the soy bean oil In fact, it was hardly possible at the same time to use margarine and butter as well as the recom-

is a Norwegian one (National Nutrition Council 1961) It is based in nearly every respect on food analyses made in various Norwegian laboratories Since this Table does not give any information on the cholesterol content of foods, or of the fatty acid composition of various fats and oils, a number of other sources have been used for such a purpose (Hayes & Rose 1957, Keys & Keys 1959, Goddard & Goodall 1959, Ensemble 1962, Grüger et al 1964, Pihl 1952)

The main data are given in Tables 11-13 It appears that the average caloric intake in the period of the study was about 2400, of which 39 per cent were fat calories Soy bean oil represented 72 per cent of the total diet fat, and in some patients marine fats were a considerable source of polyenoic fatty acids This was due to consumption of fish and cod liver oil

The data for vitamins do not include the vitamins in the tablet taken daily The requirement of vitamins seems to have been well covered. This is also the case for calcium and iron.

The calculated intake of sugar includes all sources The intake of pure sugar had been restricted. However, abundant use of marmalade, jam, fruit juice, etc had been recommended. Nevertheless, sugar consumption is low

Table 11 Cholesterol lowering diet
Daily average food intake

In parentheses calories in per cent of total calories

Pat. No	Protein g	Fat g	Total Carbohydr g	Sugar g	Alcohol (calories)	Cholesterol mg	Calories
72	75 (15)	65 (30)	271 (55)	60		175	1980
168	71 (14)	81 (36)	251 (50)	103		157	2020
51	114 (16)	123 (40)	303 (44)	22		225	2780
96	76 (17)	90 (45)	170 (39)	22		284	1790
86	108 (17)	72 (26)	358 (57)	97		222	2510
29	152 (19)	112 (32)	381 (49)	50		269	3130
31	59 (13)	81 (41)	205 (46)	22		205	1780
27	75 (12)	110 (39)	311 (49)	26		174	2540
92	86 (14)	112 (41)	270 (45)	69		148	2430
164	85 (14)	109 (41)	262 (45)	45		157	2360
20	68 (9)	170 (55)	242 (35)	70	(1)	394	2750
95	84 (16)	78 (32)	275 (52)	48		278	2120
40	106 (17)	114 (41)	265 (42)	35		356	2520
48	64 (14)	84 (43)	185 (40)	55	(3)	237	1870
181	130 (16)	156 (44)	295 (37)	38	(3)	454	3200
77	135 (17)	138 (39)	343 (43)	80	(1)	557	3170
112	80 (18)	82 (41)	188 (41)	31		199	1810
Mean of 17 pat.	92 (15)	104 (39)	269 (45.5)	51	(0.5)	264	2387

changed their food habits to some extent

Table 10 presents the results of this control of adherence to the recommended diet. There was approximately the same degree of adherence throughout the whole observation period About 60 per cent obtained the character of 'excellent' adherence, 22 per cent 'good', and 10 per cent that of 'fair' adherence Only 4 per cent got the character of 'bad' adherence The 'excellent' adherers had a mean serum cholesterol reduction of 21 6 per cent, the 'good' 12 4 per cent The 'fair' and the 'bad' adherers had a mean serum cholesterol reduction of 6 7 and 4 0 per cent respectively

Thus, there is a good accordance between the degree of adherence as judged from the answers to the questionnaires and the degree of the serum cholesterol reduction

Side effects

No serious or important side effects of the diet were registered However, a few dieters complained of light dyspeptic disorders Some reported a mildly laxative effect of the soy bean oil This effect was generally welcomed. Only three dieters had to stop or to reduce the oil consumption temporarily because of diarrhea Six dieters had short periods of nausea which disappeared when the oil consumption was reduced

The dyspeptic complaints appeared mainly in those who had the largest consumption of oil, especially when taken as such It was rarely necessary to reduce the intake of soy bean oil to less than a quarter of a liter per week

Some few dieters complained of general weakness, which they ascribed to a supposed insufficiency of the diet. These were those least motivated to make a real change in their food habits, and also those who proved to be the worst adherers These complaints were most certainly of a psychological character Clinical and laboratory examinations never revealed any cause of the complaints

As a whole, the patients in the diet group received the diet prescriptions, not with enthusiasm, but as necessary and tolerable means of improving their health The consciousness of making real efforts to improve health gave the dieters and their families an optimistic attitude with regard to the outcome of their heart disease and to the chances of keeping the ability to work.

Analysis of the diet

In order to obtain more precise information about the diet changes achieved in the diet group, 17 dieters were selected The criteria used were that they should be of the especially conscientious and positive type, and also be intelligent, so as to be able to participate in an analysis by the weighing method. The period was 7 days in 15 dieters, and 12 and 14 days in two Under the supervision of the dietician all food consumed was weighed or measured

The food composition Table used

is a Norwegian one (National Nutrition Council 1961) It is based in nearly every respect on food analyses made in various Norwegian laboratories Since this Table does not give any information on the cholesterol *content of foods, or of the fatty acid composition* of various fats and oils, a number of other sources have been used for such a purpose (Hayes & Rose 1957, Keys & Keys 1959, Goddard & Goodall 1959, Ensemble 1962, Gruger et al. 1964, Pihl 1952)

The main data are given in Tables 11-13 It appears that the average caloric intake in the period of the study was about 2400, of which 39 per cent were fat calories Soy bean oil represented 72 per cent of the total diet fat, and in some *patients* marine fats were a considerable source of polyenoic fatty acids This was due to consumption of fish and cod liver oil

The data for vitamins *do not* include the vitamins in the tablet taken daily The requirement of vitamins seems to have been well covered. This is also the case for calcium and iron

The calculated intake of sugar includes all sources The intake of pure sugar had been restricted. However, abundant use of marmalade, jam, fruit juice, etc had been *recommended* Nevertheless, sugar consumption is low

Table 11. *Cholesterol lowering diet*
Daily average food intake

In parentheses calories in per cent of total calories

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95	84 (16)	78 (32)	275 (52)	46		278	2120
40	106 (17)	114 (41)	265 (42)	35		356	2520
48	64 (14)	84 (43)	185 (40)	55	(3)	237	1870
181	130 (16)	156 (44)	295 (37)	38	(3)	454	3200
77	135 (17)	138 (39)	343 (43)	80	(1)	557	3170
112	80 (18)	82 (41)	186 (41)	31		199	1810
Mean of 17 pat.	92 (15)	104 (39)	269 (45.5)	51	(0.5)	264	2387

Table 13 *Cholesterol lowering diet - Sources of dietary fat - Fatty acid composition*

Pat No	Milk	Meat	Fish	Cereals	Vegetables Fruit	Other	Soy oil	Total fat	Sat	18 : 1	18 2	18 3	Other polyenoic
72	0 2	2 0	5 4	7.1	0 7		50	65 4	11 9	16 0	29 5	4 7	3 3
168	0 4	1 3	14 7	4 0	0 3		60	80 7	16 2	18 9	32 9	5 7	7 0
51	10 8	12 5	0 8	4 7	1 0		93	122 8	28 0	35 1	49 9	8 2	1 6
96	2 5	0 4	13 9	1 7	1.1		70	89 5	17 6	21 9	36 4	6 1	7 5
86	3 3	4 3	10 7	3 5	0 9		48	71 7	16 9	18 3	27 1	4 5	5 9
29	1 5	0	30 2	8 8	0 9	1 0	70	112 4	23 5	25 5	41 1	7 1	15 2
31	1 7	1.3	10 2	4 2	0 7	1 0	62	81 1	15 9	20 1	33 9	5 7	5 5
27	3 6	4 0	9 1	6 3	1 7		85	109 7	21 5	27 9	46 7	7 6	6 0
92	1 8	2 2	2.5	5 6	0 2	1 7	100	112 3	20 5	28 8	53 0	8 6	1 4
154	4 2	5.3	9 4	7 4	0 5		82	108 9	22 3	28 0	45 8	7 5	5 1
20	33 2	20 2	6 0	11.2	0 7	28 4	70	169 6	51 3	50 8	46 8	7 5	10 2
95	4 0	3.2	4 0	3 3	0 5		63	78 0	15 7	20.5	33 7	5 6	2 5
40	0 6	1 9	8 3	3 6	0 9		99	114 3	20 3	28 8	52 0	8 5	4 7
48	0 3	10 0	3 6	2 7	0 2	2 0	65	83 8	17 7	23 5	34 8	5 8	2 0
181	4 6	5 5	41 7	4 7	0 6		99	156 1	35 6	38 0	53 6	9 4	19 5
77	4 5	0 6	29 3	6 3	0 8		96	137 5	28 8	32 8	52 6	9 1	14 2
112	1 0	4 6	6 9	2 7	0 7		66	81 9	15 9	21 4	34 9	5 8	3 9
Mean of 17 pat.	4 6	4 6	12 1	5 2	0 7	2 0	75	101.2	22 5	26 8	41.3	7 0	6 6
Per cent of total fat	4 4	4 4	11 6	5 0	0 7	1 9	72 0	100	21 6	25 7	30 7	6 7	6 3

Table 12 *Cholesterol lowering diet*
Daily intake of calcium, iron and vitamins (the tablet vitamins not included)

		Mean of 17 pat.	Range
Calcium (mg)		996	436 1876
Iron (mg)		12	7 18
Vit. A (IU)		7787	800-21770
Thiamine (mg)		1 4	0.8-2 1
Riboflavin (mg)		2 0	1.2-4.0
Niacin (mg)		20 1	35 2 11 3
Vit C (mg)	raw	87	29 165
	served	63	23 126
Vit. D (IU)		610	31 2367

Table 14 presents the effect on the body weight of the analyzed diet. At the time of analysis, 5 of the 17 dieters had gained weight, and 11 had lost weight. There was an average reduction of body weight of 2 6 kg, which is almost the same as for the whole observation time in these dieters (2 7 kg) The main reduction of weight took place in the four dieters who at the start of the trial were overweight, their mean reduction being 9 4 kg

In Table 15 the effect of the ana

Table 14. *Cholesterol lowering diet*
Effect on weight

Pat. No	Height (cm)	Weight at start (kg) (Deviation from normal weight)		Weight change at analysis (kg)	Mean weight change during obs. time (kg)
72	165	66 7	(0)	– 2.2	–2.0
168	170	79.2	(+ 15 %)	–10 7	–7.8
51	178	77.9	(0)	– 5.3	–4.8
96	169	72 7	(0)	+ 1 1	–2.3
86	182	83 0	(+ 10 %)	– 9 9	–5.3
29	172	59 0	(–10 %)	– 0 4	+ 1.5
31	162	59.5	(0)	0	–3 0
27	180	73 7	(0)	– 0 7	–2.1
92	169	61 6	(0)	+ 4 1	+ 0 6
154	167	66.8	(0)	– 7 3	–5 0
20	163	63 0	(0)	+ 2 0	+ 0 6
93	171	71 8	(0)	+ 3.5	+ 4.1
40	170	71 5	(0)	– 0 1	–0.5
48	170	70 8	(0)	+ 0 6	–1.8
181	165	65.2	(0)	– 2.1	–4.8
77	167	77 5	(+ 15 %)	– 4 6	–3.5
112	173	90 1	(+ 30 %)	–12.3	–9 5
Mean of 17 pat.	170	71 2	(0)	– 2 6	–2 7

Deviation from normal weight
0 ≈ ± 10 % of normal weight of Norwegian men
+ 10 % ≈ 11 15 % above » » » » »
+ 15 % ≈ 16 20 % » » » » » »
+ 30 % ≈ 31 35 % » » » » » »
– 10 % ≈ 11 15 % below » » » » »

lyzed diet on the serum cholesterol concentration is presented. The mean reduction in these 17 dieters is 31 per cent, which is very much the same as for the whole trial (for these dieters, 29 per cent)

Table 15 *Cholesterol lowering diet*
Effect on serum cholesterol

Pat. No	Cholesterol at start (mg/100 ml)	Cholesterol at diet analysis (mg/100 ml)	Cholesterol at diet analysis (per cent reduction)	Mean chol. reduction during obs time (per cent)
72	259	145	44	34
168	262	147	44	40
51	243	149	39	30
96	333	290	13	17
86	322	183	43	42
29	256	186	27	16
31	520	249	52	43
27	403	300	26	27
92	208	176	15	27
154	311	280	10	23
20	287	181	37	19
95	234	189	19	21
40	260	187	28	35
48	284	154	46	32
181	252	197	22	32
77	249	196	21	26
112	320	193	40	35
Mean of 17 pat.	294	200	31	29

In conclusion, three main principles sharply distinguish the experimental diet from conventional food habits in Norway, as outlined in the next chapter The diet is low in saturated fats, high in polyunsaturated fats, and low in dietary cholesterol

Further, a mean serum cholesterol reduction of 31 per cent at the time of diet analysis as compared with 29 per cent for the whole trial in these dieters demonstrates a strong cholesterol-reducing effect of the diet and that it was possible to adhere to the prescribed diet for a considerable length of time

Chapter 4

FOOD HABITS IN THE CONTROL GROUP

Since the food habits of Norwegians may be largely unknown in other countries, a brief description of the food habits in Oslo is warranted.

These are relatively uniform, although economic class to some extent may influence the choice of food Three to four meals a day are the routine, with one hot meal, dinner at 4 6 p.m The other meals are of the bread and butter type with cheese, jam, meat, etc, spread on bread. The average egg consumption is half an egg per head per day Milk consumption is high Dinner generally is a two-course meal. Meat or fish is the staple food, served with boiled potatoes Soup or dessert is the other course Vegetables and fruit are consumed according to season and supply

Information on the food consumption in Norway for the years 1953 63 can be found in Food Consumption in OECD countries (O E C D, Paris 1963)

Analysis of the food composition by Øgrim & Homb (1960), based on surveys carried out by the Central Bureau of Statistics for the years 1947-54 (industrial workers and employees), showed that 40 per cent of the calories were derived from fat, 12 from protein, and 48 from carbohydrates Per head of the population the daily fat consumption is about 130 g, of which 65 g is margarine, the rest chiefly animal fats Only 2-4 per cent of the fat calories are represented by fish fat. Nearly all marine fat used for human consumption, 40-50 g per day per head, is hydrogenated and used in the manufacture of margarine

The intake of polyenoic acids, mainly linoleic acid, has been approximately constant since 1945-50 According to calculations by Eeg Larsen (1963, 1964), the daily intake per 3,000 calories has been 8.5—9 0 g

According to official statistics (Royal Norwegian Department of Agriculture 1961) fats were used in the manufacture of margarine in approximately the following proportions hydrogenated marine oils 68, hydrogenated vegetable oils 2, coconut fat 14, and vegetable oil 11. Margarine is manufactured by a considerable number of factories, and the

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Information on the food consumption in Norway for the years 1953-63 can be found in Food Consumption in OECD countries (O E C.D, Paris 1963)

Analysis of the food composition by Øgrim & Homb (1960), based on surveys carried out by the Central Bureau of Statistics for the years 1947-54 (industrial workers and employees), showed that 40 per cent of the calories were derived from fat, 12 from protein, and 48 from carbohydrates Per head of the population the daily fat consumption is about 130 g, of which 65 g is margarine, the rest chiefly animal fats Only 2-4 per cent of the fat calories are represented by fish fat. Nearly all marine fat used for human consumption, 40 50 g per day per head, is hydrogenated and used in the manufacture of margarine

The intake of polyenoic acids, mainly linoleic acid, has been approximately constant since 1945-50 According to calculations by Eeg Larsen (1963, 1964), the daily intake per 3 000 calories has been 8 5—9 0 g

According to official statistics (Royal Norwegian Department of Agriculture 1961) fats were used in the manufacture of margarine in approximately the following proportions hydrogenated marine oils 68, hydrogenated vegetable oils 2, coconut fat 14, and vegetable oil 11. Margarine is manufactured by a considerable number of factories, and the

lyzed diet on the serum cholesterol concentration is presented The mean reduction in these 17 dieters is 31 per cent, which is very much the same as for the whole trial (for these dieters, 29 per cent)

Table 15 *Cholesterol lowering diet*
Effect on serum cholesterol

Pat. No	Cholesterol at start (mg/100 ml)	Cholesterol at diet analysis		Mean chol. reduction during obs time (per cent)
		(mg/100 ml)	(per cent reduction)	
72	259	145	44	34
168	262	147	44	40
51	243	149	39	30
96	333	290	13	17
86	322	183	43	42
29	256	186	27	16
31	520	249	52	43
27	403	300	26	27
92	208	176	15	27
154	311	280	10	23
20	287	181	37	19
95	234	189	19	21
40	260	187	28	35
48	284	154	46	32
181	252	197	22	32
77	249	196	21	26
112	320	193	40	35
Mean of 17 pat.	294	200	31	29

In conclusion, three main principles sharply distinguish the experimental diet from conventional food habits in Norway, as outlined in the next chapter The diet is low in saturated fats, high in polyunsaturated fats, and low in dietary cholesterol

Further, a mean serum cholesterol reduction of 31 per cent at the time of diet analysis as compared with 29 per cent for the whole trial in these dieters demonstrates a strong cholesterol-reducing effect of the diet and that it was possible to adhere to the prescribed diet for a considerable length of time

Table 16 *Food habits in control group*

Degree of changes of food habits	N	Per cent	Mean cholesterol reduction (per cent)
Score ≥ 42	0		
» 34-41	3	2 1	8 0
» 25-33	7	4 7	7 0
» < 25	138	93.2	2 4

Mean score 12 9 points

A mean score of 12 9 points indicates that certain diet changes had also occurred in the control group However, the type of score used was such as gave certain values to alterations in choice of food occurring normally in an adult population. Thus, leaving out whole milk and cream from the daily diet gave a score of 12 points, regardless of the composition of the remaining diet.

The difference between the food habits of the two groups is well illustrated by the consumption of butter or margarine and of edible oils At the last examination there were 142 (96 per cent) in the control group who used butter or margarine, and 130 (88 per cent) who not did use vegetable oils in any form. In contrast, in the diet group only 21 (14 per cent) used butter or margarine, and only 6 (4 per cent) did not use soy bean oil

The difference in diet habits between the two groups was even greater than these figures may indicate Being restricted food, the consumption of butter or margarine in the 21 dieters was very small, usually scraped on a few slices of bread On the other hand, the oil consumption of the 18 controls was very small, the oil being mainly used for frying and in sauces, and thus far less than that used by the dieters

Although not knowing the exact composition of the diet of the control patients, the opinion that their diet did not depart far from conventional Norwegian food habits seems well founded. The results of the serum cholesterol study strongly support this contention

composition of all varieties is not known However, one margarine in common use covers about 30-40 per cent of all margarine sold in Oslo According to information from the manufacturer, in the autumn of 1961 soy bean oil was substituted for peanut oil in this margarine This would increase the content of polyenoic fatty acids by about 3 g per 100 g of margarine

The food habits in the control group were presumably influenced by the general professional and lay attitude towards CHD, which varied to some extent during the years of the trial

At the start of the trial, as indicated by the high percentage in this trial given long-term anticoagulant treatment (Table 2), such therapy was common in Oslo Later in the trial, national and international discussion on diet and CHD, with emphasis on restriction of total fat consumption and some substitution of polyunsaturated fats for saturated ones, undoubtedly had some influence with regard to dietary measures advocated and used, not only by patients who already had had an attack, but also by other middle-aged men

For a brief period of about one year the publicity about the possible relation between diet and CHD largely shifted to the opinion that the disease might be caused by a pure linolenic acid deficiency (Owren et al 1964) Further, the observed associations between the prevalence of CHD and various conditions such as dietary sugar (Yudkin 1957, 1964), the hardness of the drinking water, physical activity, etc, and the appearance of several cholesterol-reducing agents, such as thyroid preparations, triparanol, atromid, etc, worked in the same direction, and added to the hesitation towards dietetic preventive measures against CHD

Thus, it occurred that, with regard to the control group, the present trial benefited by the different views on the etiology, and by the changing opinion of the means of prevention of CHD held by medical and nutritional authorities

I based the contact with the control patients on medical follow-up of their heart disease Direct questions about the diet were answered honestly, but the doubt and the different opinions held by authorities were always stressed From my personal contact with the control patients, I formed the opinion that some of them had introduced some moderation with regard to fat intake, and some few had also started to use moderate quantities of oils However, the general impression obtained in the years of very close contact was that the patients in the control group, except for a few, continued on their habitual diet.

At the end of the 5-year period, the 148 controls still alive were examined closely with regard to details of the diet. The questionnaire and the type of score used were the same as those used in the diet group Table 16 gives the results, which contrast strikingly with those given for the diet group in Table 10

244 mg per 100 ml against 285 in the control group This difference was in the main obtained during the first three months of the trial From then on the serum cholesterol curves run parallel (Fig 1)

High initial serum cholesterol values were more reduced than the medium and low However, the difference between the groups was the same, irrespective of the start level (Figs 2 and 3)

The percentages of patients on different serum cholesterol levels also differed during the whole observation period in the two groups (Table 17)

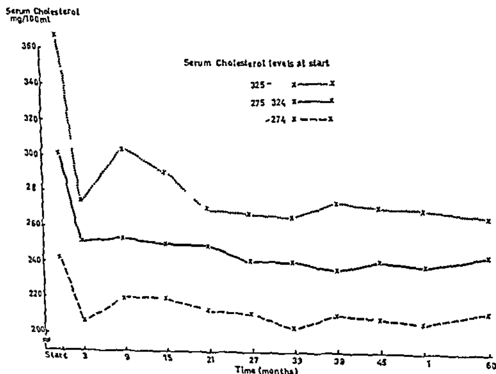


Figure 2 Diet group Serum cholesterol levels during the observation time.

Start level	N (at start)	Start value (mg/100 ml)	Mean reduction (mg)	(per cent)
325-	51	367	90	24.5
275-324	82	301	55	18.2
274	73	243	26	10 7

Chapter 5

SERUM CHOLESTEROL LEVEL DURING OBSERVATION TIME

The results for the two groups are presented in Figs 1-4 and Tables 17-19 The averages represent the results in the patients examined at the given intervals as is evident from Tables 4 and 5 Since death occurred more frequently in the control group, and also more frequently in patients with high than with low serum cholesterol, the means at different times are not strictly comparable

At the start of the trial the mean serum cholesterol value and the distribution at different cholesterol levels were the same in the two groups as shown previously (Table 2 III 0)

From the first examination, 3 months after the start of the trial, and at each of the ten examinations throughout the 5-year observation, there was a significant difference between the two groups, the mean cholesterol value in the diet group being

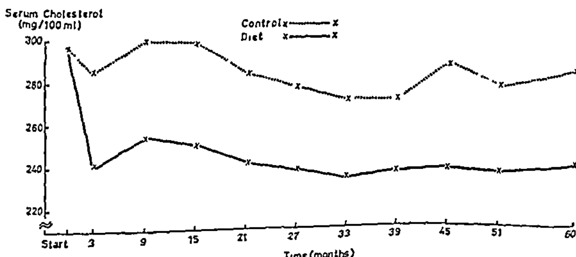


Figure 1 Serum cholesterol levels in the diet and the control group during the observation time

	N (at start)	Start value (mg/100 ml)	Mean reduction (mg)	(per cent)
Control	206	296	11	3 7
Diet	206	296	52	17 6

The group averages of the controls indicate that their serum cholesterol remained largely unchanged. If data for the survivors only are used for comparison, this conclusion is well substantiated (Table 18) The values at start and at 3 months later for these 148 controls were 292 and 284 mg per 100 ml respectively, as compared with 276 and 283 at 51 and 60 months respectively Thus the group average at 3 and 60 months after start was identical for the 148 surviving controls regularly examined. Such a remarkable constancy of serum cholesterol in a group of 148 over a period of 5 years seems to suggest quite strongly that the changes in the diet with regard to quantity and composition of the fats consumed had been too small to influence the serum cholesterol level in the group as such

Table 18 *Serum cholesterol values in the survivors, regularly controlled during 5 years*

	Control	Diet
Start	292	298
3 months	284	244
9 »	297	252
15 »	298	248
21 »	282	239
27 »	278	235
33 »	270	231
39 »	271	235
45 »	288	238
51 »	276	235
60 »	283	239

On the other hand, more detailed data given in Fig 4 and Table 19

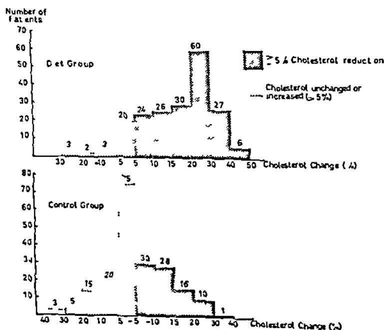


Figure 4. Frequency distribution of serum cholesterol changes.

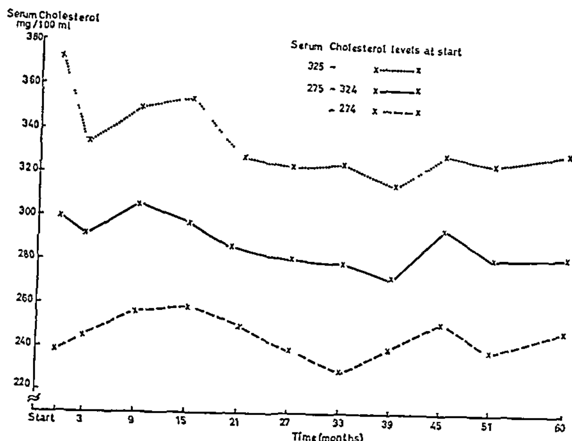


Figure 3 Control group Serum cholesterol levels during the observation time.

Start level	N (at start)	Start value (mg/100 ml)	Mean change (mg)	Mean change (per cent)
325-	56	372	−37	−9 9
275-324	74	299	−12	−4 0
-274	76	238	+ 8	+ 3 4

Table 17 *Percentage of patients with different serum cholesterol levels in the diet and the control group at various points during the observation time*

Cholesterol mg/100 ml		Start Diet	Start Control	15 months Diet	15 months Control	27 months Diet	27 months Control	45 months Diet	45 months Control	60 months Diet	60 months Control	Mean (start value excluded) Diet	Mean (start value excluded) Control
-199		1 4	2 9	16 1	3 1	22 6	8 1	22.2	3 8	18 7	6 2	19 9	5.3
200-249		17 3	17 5	37.3	20 7	38.2	27 2	37 7	23.3	41 7	24 1	38 7	23 8
250-299		33 7	35 4	30 6	30 1	25 8	33 7	31 5	34 6	27 7	35 7	28 9	33 5
300-349		31 7	29 1	9 8	26 9	10 2	19 6	6 8	22 0	10 6	20 2	9 3	22 2
350-		15 9	15 1	6.3	19 2	3 2	11 4	1 8	16 3	1.3	13 8	3.2	15 2
Mean chol value	All	296	296	251	299	238	277	238	288	239	283	244	285
	Survivors	298	292	248	298	235	278	238	288	239	283	240	283

The group averages of the controls indicate that their serum cholesterol remained largely unchanged. If data for the survivors only are used for comparison, this conclusion is well substantiated (Table 18) The values at start and at 3 months later for these 148 controls were 292 and 284 mg per 100 ml respectively, as compared with 276 and 283 at 51 and 60 months respectively Thus, the group average at 3 and 60 months after start was identical for the 148 surviving controls regularly examined Such a remarkable constancy of serum cholesterol in a group of 148 over a period of 5 years seems to suggest quite strongly that the changes in the diet with regard to quantity and composition of the fats consumed had been too small to influence the serum cholesterol level in the group as such.

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On the other hand, more detailed data given in Fig 4 and Table 19

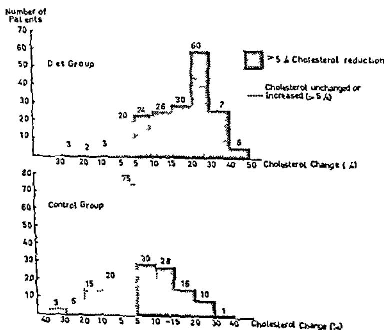


Figure 4. Frequency distribution of serum cholesterol changes.

indicate that the averages of the control group are not fully representative, since a 10-15 per cent cholesterol reduction occurred in 28, and ≥ 15 per cent reduction in 27 Since the corresponding percentages of increase are distinctly smaller, it ma[y] be assumed that in some of the controls reduction was due to spontan[e]eous variations, and in some it wa[s] due to the reduction known to occu[r] in men over 60 years of age.

Table 19 *Frequency distribution of serum cholesterol changes according to cholesterol level at start (mean value in per cent of start value)*

	Serum cholesterol at start: Level	No of pat.	Increase: > +40	+40 – +30	+30 – +20	+20 – +10	+10 – +5	+5 – −5	Reduction: −5 – −10	−10 – −15	−15 – −20	−20 – −30	−30 – −40	> −40	Cholesterol reduction: No of pat.	Per cent of pat.
Diet group	-249	34				2	1	3	6	4	7	11			28	82 4
	250 299	70			3		2	13	11	8	10	16	6	1	52	74.3
	300-349	66						2	6	11	9	23	13	2	64	97 0
	350-	31						2	1	3	4	10	8	3	29	93 5
	Total	201			3	2	3	20	24	26	30	60	27	6	173	80.2
Control group	-249	42			3	5	3	20	9	2					11	26.2
	250-299	73	1	1	1	7	11	28	8	7	7	2			24	32 9
	300-349	57			1	1	5	19	10	12	5	4			31	54 4
	350-	31		1		2	1	8	3	7	4	4	1		19	61.3
	Total	203	1	2	5	15	20	75	30	28	16	10	1		85	41 9

The data in Table 19 also indicate that the patients in the control group with the highest levels of serum cholesterol in reality altered their diet, as was also evident from the examination of food habits of the controls (Table 16)

However, a comparison with the serum cholesterol in the diet group makes it clear that the aim was achieved, namely a substantial reduction of the serum cholesterol level in the dieters as compared with the controls

While 27 (13 3 per cent) of the controls reduced their serum cholesterol by ≥ 15 per cent, this reduction was achieved in 123 dieters (61 1 per cent) While about three-quarters of the dieters achieved a ≥ 10 per cent cholesterol reduction, about three-quarters of the controls kept their serum cholesterol at a 'satisfactory' level (< 10 per cent reduction) during the observation time

Chapter 6

RESULTS

Myocardial reinfarction

Table 20 presents the incidence of myocardial reinfarction in the two groups during the trial In the diet group 34 patients had 43 reinfarcts 10 of which were fatal. In the control group 54 patients had 64 reinfarcts, 23 of which were fatal The difference between the groups is statistically significant, both with respect to the total number of patients with reinfarction ($x^2 = 5.22$, $p = 022$), and the number with fatal infarctions ($x^2 = 4\ 74$, $p = 029$)

Table 21 presents the autopsy result. Fibrotic changes of old myocardial infarctions were found in all cases The typical changes of fresh myocardial infarcts were found both macroscopically and microscopically in all the cases in the diet group, and in all but four cases in the control group *In the latter four cases* a definite clinical diagnosis of myocardial reinfarction could be made in two (diagnostic board), and the remaining two cases will be presented as case reports below (C-80 and C-186) Fresh thrombus formation was found in 4 of the 9 fatal cases in the diet group, and in 15 of the 21 control cases in which autopsy was performed.

Table 20 *Incidence of myocardial reinfarction*

		Diet group				Control group			
Type *		Pat.	Reinfarct.	Deaths	Autopsy	Pat.	Reinfarct.	Deaths	Autopsy
I	Clinical diagnosis made	25	28	1	0	35	42	4	2
I + II	Survived clinical, later fatal reinfarction verified by autopsy	3	9	3	3	3	6	3	3
II	One reinfarction only verified by autopsy	6	6	6	6	14	14	14	14
III	Fatal episodes with chest pains. Autopsy made	0	0	0	0	2	2	2	2
	Total reinfarctions	34	43	10	9	54	64	23	21

* Definitions on p 20

Table 21 *Myocardial reinfarction*
Autopsy results

	Diet group		Control group	
	Present	Not present	Present	Not present
Old myocardial infarction	9	0	21	0
Fresh » »	9	0	17	4
Fresh coronary thrombus	4	5	15	6
Rupture of interventricular septum			2	

Case reports

C-80, born 1906 March 1957 treated in hospital for a first myocardial infarction Subsequent peroral anticoagulant treatment for one year Start of observation time, 10 February 1959 No angina

Admitted to hospital 19 May 1961 after having sustained heavy chest pains for 3 hours. On admission only moderate pains and in relatively good state Died suddenly before any laboratory tests had been taken

Autopsy Aorta moderate atheromatosis Heart weight 490 g No ventricular hypertrophy Normal valves and ostia. No thrombi in the atria or ventricles Moderate coronary atheromatosis, except from an area 4 cm distally to the start of the right coronary artery The lumen here was totally occluded by atheromatosis with a fresh thrombus formation distally In the myocardium an old infarction in the posterior part of the left ventricle and in the interventricular septum but no fresh infarct was found

Other organs nothing noteworthy

C-186, born 1896 October 1958 treated in hospital for a first myocardial infarction. Discharged from the hospital with long term peroral anticoagulant treatment. Admitted to the controlled trial on 26 January 1960 Angina pectoris on effort, relieved by nitroglycerine.

Admitted to hospital 11 October 1960 after hav[illegible] pains for 6 hours, not relieved by nitroglycerine On admission in cardiogenic shock, and complaining of heavy chest pains. Died while being examined by the doctor, before any laboratory tests had been performed

Autopsy Aorta pronounced atheromatosis with ulcerations *(degree IV)* Heart weight 480 g Significant hypertrophy of the left ventricle, moderate hypertrophy of the right ventricle. Normal valves and ostia No thrombus formation *in the* atria or in the ventricles Opening of the coronary arteries with scissors revealed no definite fresh thrombi. Both coronary arteries were practically totally occluded and it was difficult to find any *lumen. In the* posterior part of the left ventricle an old infarction was found. In the interventricular septum color changes suspect of a fresh infarct. Microscopy No definite fresh infarct.

Lungs Abundant edema

Other organs nothing noteworthy

Acquired angina pectoris

At the start of the trial and at each further examination, the patients were thoroughly examined for symptoms of angina pectoris In the patients with previous angina, changes in the severity of symptoms were also registered However, proper evaluation of improvment or deterioration of angina pectoris being more

Table 22. *Angina pectoris at start of observation and at last examination*

Angina	Diet group: Start of observation	Last examination	Difference	Control group: Start of observation	Last examination	Difference
Not present	75	64 + 1 ?	10	79	49 + 1 ?	29
Present	122	105 + 1 ?	16	119	110	9
Uncertain	9	3	6	8	2	6

difficult to estimate than the mere presence or absence of the angina pectoris syndrome, it was decided only to judge if at the last examination they had manifest angina on physical or emotional effort or not.

Table 22 presents the results of this examination At the start of the trial 75 in the diet group and 79 in the control group were without angina pectoris During the observation period, 10 (13 3 per cent) of the dieters and 29 (36 7 per cent) of the controls acquired angina pectoris The difference is statistically significant ($x^2 = 9\ 92$, p = 002)

Of the patients with definite angina at the start, 16 (13 1 per cent) of the dieters and 9 (7 6 per cent) of the controls got rid of their angina during the observation time

Sudden death

Table 23 presents the incidence of sudden death according to definitions previously given (p 20) There is no difference between the groups, 27 being listed in each group In type

Table 23 *Incidence of sudden death, including unexplained, probably coronary death*

Type *		Diet group: N	Diet group: Autopsy	Control group: N	Control group: Autopsy
I	Instantaneous death	18	5	13	3
II	Chest pains preceding death	1	0	8	0
III	Unwitnessed death	8	1	6	3
	Total	27	6	27	6

* Definition on p 20

Table 24. *Sudden death*
Autopsy results

	Diet group: Present	Diet group: Not present	Control group: Present	Control group: Not present
Old myocardial infarction	6	0	6	0
Fresh myocardial infarction	0	6	0	6
Fresh coronary thrombus	1	5	0	6
Aortic stenosis	1			

Table 21 *Myocardial reinfarction*
Autopsy results

	Diet group		Control group	
	Present	Not present	Present	Not present
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Fresh » »	9	0	17	4
Fresh coronary thrombus	4	5	15	6
Rupture of interventricular septum			2	

Case reports

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Present	122	105 + 1 ?	16	119	110	9
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difficult to estimate than the mere presence or absence of the angina pectoris syndrome, it was decided only to judge if at the last examination they had manifest angina on physical or emotional effort or not.

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Table 24. *Sudden death*
Autopsy results

	Diet group: Present	Not present	Control group: Present	Not present
Old myocardial infarction	6	0	6	0
Fresh myocardial infarction	0	6	0	6
Fresh coronary thrombus	1	5	0	6
Aortic stenosis	1		0	6

I of sudden death, instantaneous death, there are 18 in the diet group and 13 in the control group, and in type III, unwitnessed death, the figures are 8 and 6 However, in type II, fatal episodes with chest pains of minutes to few hours duration preceding death, there is only one in the diet group against 8 in the control group

Table 24 presents the autopsy results In all cases an old myocardial infarct was found Fresh infarct was found in none Fresh coronary thrombus formation was found in one case in the diet group, and aortic stenosis also in one case in the diet group

Total CHD mortality

The total CHD mortality — fatal myocardial infarction and sudden death — is 37 in the diet group and 50 in the control group The difference is not statistically significant (x^2 = 2 10, p = 15)

Including the two cases of fatal cerebral stroke and the case of fatal aortic thrombosis, the figures for the total cardiovascular mortality are 38 in the diet group, and 52 in the control group

Total CHD relapses

Table 25 presents the total incidence of CHD relapses in the two groups The total number of relapses is 80 in the diet group and 120 in the control group The total number of patients with CHD relapses is 64 in the diet and 90 in the control group The difference is statistically significant (x^2 = 6 48, p = 011)

As the incidence of sudden death was the same in the two groups, the difference between the diet group and the control group with regard to the combined relapses of myocardial infarction and acquired angina pectoris was even greater, the number of patients being 42 in the diet group and

Table 25 *Total incidence of CHD relapses*

A Total number of CHD relapses

	Diet group	Control group
Myocardial reinfarctions	43	64
Acquired angina pectoris	10 (also reinfarction 2)	29 (also reinfarction 9)
Sudden death	27 (also reinfarction 4) (also acq angina 1)	27 (also reinfarction 7) (also acq angina 4)
Total	80	120

B Total number of patients with CHD relapses

	Diet group	Control group	P value
Myocardial reinfarction	34	54	022
Acquired angina pectoris	8	20	031
Sudden death	22	16	> 05
Total	64	90	011

74 in the control group The difference is highly significant (χ^2 = 11 53, p = 001)

Comments

In the type III reinfarction cases (p 20), case reports have been given (p 44) The reason these two cases, in which heavy and lasting chest pains preceded death, are also regarded as reinfarctions in spite of the fact that no definite clinical or autopsy diagnosis could be made is that autopsy failed to reveal any other cause of the heavy chest pains which preceded death, such as a ruptured aortic aneurysm or pulmonary emboli The absence of positive changes of a fresh infarction is consistent with the short duration of the supposed myocardial infarction episode In one of these cases (C-80) a fresh coronary thrombus was found, and in the other (C-186) both the coronary arteries were practically totally occluded by atheromata, making it difficult to find a possible fresh thrombus formation

The type II cases of sudden death (p 20) were not regarded as myocardial reinfarctions although chest pains also preceded death because autopsy was *not performed* Thus it was impossible to exclude other causes of death preceded by chest pains However, these patients who had survived a myocardial infarction and now sustained heavy chest pains for minutes to hours before death most probably had a new coronary occlusion.

If, therefore, the type II cases of *sudden death are also regarded as* myocardial infarctions, the number *of patients* with reinfarction will be 34 in the diet group, and 60 in the control group (The one in the diet group and two of the eight controls had previously had a reinfarction) The difference between the groups with regard to the incidence of reinfarction will then be even more significant (χ^2 = 8 62, p = 003)

In the diagnosis of acquired angina pectoris a personal bias may have been present. This diagnostic task might also have been left to the diagnostic board. This was not done due to the fact that the diagnosis of angina pectoris must be based on a personal examination of the patient, *and in many instances only after* repeated examinations Thus, it would have been impractical to leave this time-consuming work to the diagnostic board. On the other hand, having known each patient personally for years, I was in a favorable position to decide if the patient's symptoms really were angina pectoris or not.

Sudden death is the most common cause of death in the present material All died outside hospitals except for 2 in the diet group and 3 in the *control group In none of these cases* was monitoring or terminal electrocardiography performed which might have revealed the mechanism of death

In the type I cases patients dying instantaneously, a so-called electrical failure resulting in ventricular fibrillation, or heart stand still, most

I of sudden death, instantaneous death, there are 18 in the diet group and 13 in the control group, and in type III, unwitnessed death, the figures are 8 and 6 However, in type II, fatal episodes with chest pains of minutes to few hours duration preceding death, there is only one in the diet group against 8 in the control group

Table 24 presents the autopsy results In all cases an old myocardial infarct was found Fresh infarct was found in none Fresh coronary thrombus formation was found in one case in the diet group, and aortic stenosis also in one case in the diet group

Total CHD mortality

The total CHD mortality — fatal myocardial infarction and sudden death — is 37 in the diet group and 50 in the control group The difference is not statistically significant ($x^2 = 2\ 10$, $p = \ 15$)

Including the two cases of fatal cerebral stroke and the case of fatal aortic thrombosis, the figures for the total cardiovascular mortality are 38 in the diet group, and 52 in the control group

Total CHD relapses

Table 25 presents the total incidence of CHD relapses in the two groups The total number of relapses is 80 in the diet group and 120 in the control group The total number of patients with CHD relapses is 64 in the diet and 90 in the control group The difference is statistically significant ($x^2 = 6\ 48$, $p = \ 011$)

As the incidence of sudden death was the same in the two groups, the difference between the diet group and the control group with regard to the combined relapses of myocardial infarction and acquired angina pectoris was even greater, the number of patients being 42 in the diet group and

Table 25 *Total incidence of CHD relapses*

A Total number of CHD relapses

	Diet group	Control group
Myocardial reinfarctions	43	64
Acquired angina pectoris	10 (also reinfarction 2)	49 (also reinfarction 9)
Sudden death	27 (also reinfarction 4) (also acq angina 1)	27 (also reinfarction 7) (also acq angina 4)
Total	80	120

B Total number of patients with CHD relapses

	Diet group	Control group	P value
Myocardial reinfarction	34	54	022
Acquired angina pectoris	8	20	031
Sudden death	22	16	> 05
Total	64	90	011

Chapter 7

CHD RELAPSES IN RELATION TO VARIOUS FACTORS

1 Age

The data for the CHD relapse incidence in relation to age have been given in Figs 5-7 and in Table 26

Both below the age of 50 and of 60 (Fig 5) the total CHD relapse incidence in the diet group was significantly lower than in the control group (x^2 = 4 30, p = 038 and x^2 = 4 91, p = 027 respectively) At the age ≥ 60, there was not a significant difference between the groups with regard to the total CHD relapse rate (x^2 = 0 86, p = .35)

Below the age of 60 (Fig 6) the combined relapse rate of myocardial reinfarction and of angina pectoris was significantly lower in the diet than in the control group (x^2 = 6 65, p = 010) At the age ≥ 60 (Fig 7) this combined relapse rate did not dif-

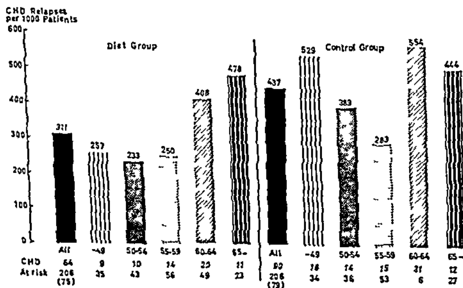


Figure 5 Incidence of total CHD relapses in relation to age. In parentheses number at risk of acquiring angina pectoris.

probably occurred Autopsy was performed in 8 of these cases, showing an old myocardial infarct and a varying degree of coronary atheromatosis in all cases, but in none a fresh myocardial infarct.

The type II cases have been discussed together with myocardial reinfarction, which is the probable cause of death in these cases, although it is not proven

In the type III cases, unwitnessed death, most probably some died instantaneously, as in the type I cases, and some may have had chest pains before death as in the type II cases. Autopsy was performed in 4 of these cases, showing an old myocardial infarct and a varying degree of atherosclerosis in all cases, but failing to show fresh coronary occlusion or fresh myocardial infarct.

Table 26 *Total CHD mortality in relation to age*

	Age < 60		Age ≥ 60	
	Diet	*Control*	*Diet*	*Control*
At risk	134	123	72	83
Fatal myocardial reinfarction	5	10	5	13
Sudden death	13	13	14	14
Total CHD mortality	18	23	19	27

fer significantly on the five per cent level, although the difference was quite large (x^2 = 3 56, p = 060)

With regard to the total CHD mortality (Table 26) there was still no statistically significant difference between the groups even after the splitting into age subgroups There was, however, a strong trend towards a lower mortality in the diet group

2 Time

The data relative to the time in the trial at which the various CHD relapses occurred have been given in Figs 8-13 by means of cumulative numbers of patients who had suffered relapses at the end of each year

The difference between the groups in the incidence of myocardial reinfarction (Figs 8 and 9) was small during the first two years However, during the third year the difference increased both with regard to the total number of patients with reinfarction and to the number of fatal reinfarctions During the fourth year the difference between the groups became statistically significant.

As seen in Fig 10 the difference between the groups with regard to the incidence of new cases with angina pectoris was pronounced during the whole trial.

The incidence of sudden death (Fig 11) was the same during the whole observation period. However, with regard to the total CHD mortality (Fig 12), owing to the increased reinfarction mortality in the control group, there was a difference between the two groups during the trial although it was not statistically significant on the five per cent level

Fig 13 presents the total number of patients who suffered CHD relapses during the course of the trial. As previously stated, only one relapse is counted for each patient, the order of priority being reinfarction, acquired angina pectoris, and sudden death

There was an increasing difference between the groups in favor of the dieters from the very start of the trial The difference was not statistically significant during the first two years However, from the second year the difference increased, and for the rest of the observation period the dieters had a statistically significantly lower total CHD relapse rate.

This course of the CHD relapses is

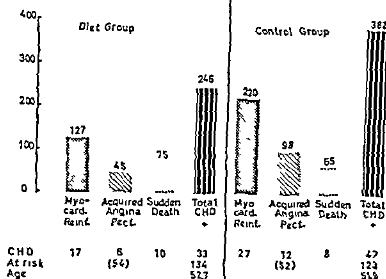


Figure 6 Age < 60 Incidence of CHD relapses
In parentheses number at risk of acquiring angina pectoris.

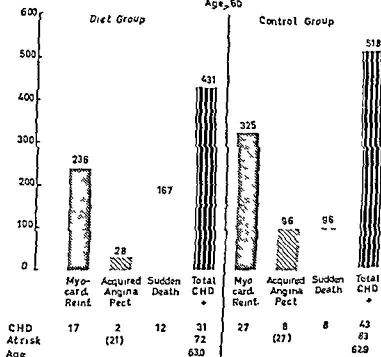


Figure 7 Age ≥ 60 Incidence of CHD relapses.
In parentheses number at risk of acquiring angina pectoris.

Table 26 *Total CHD mortality in relation to age*

	Age < 60		Age ≥ 60	
	Diet	Control	Diet	Control
At risk	134	123	72	83
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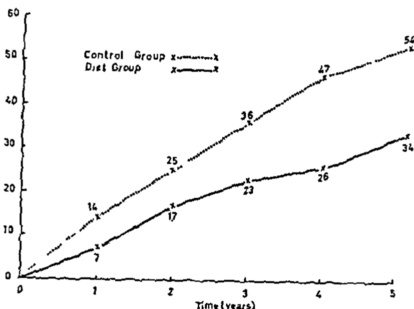


Figure 8 Cumulated incidence of myocardial reinfarction.

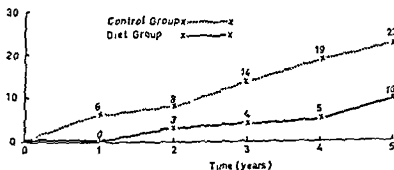


Figure 9 Cumulated incidence of fatal myocardial reinfarction

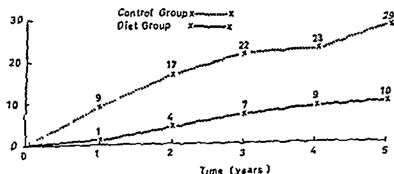


Figure 10 Cumulated incidence of acquired angina pectoris

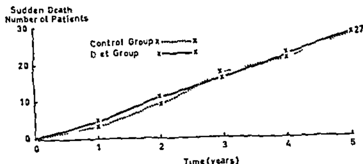


Figure 11 Cumulated incidence of sudden death.

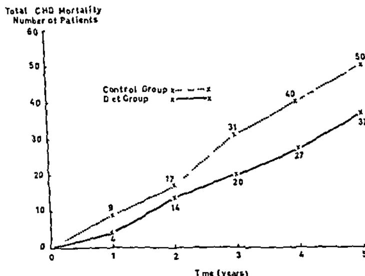


Figure 12 Cumulated incidence of total CHD mortality (fatal reinfarction and sudden death)

in contrast to the results of controlled trials of the effect of long term anti coagulant treatment in myocardial infarction. Bjerkelund (1957), for example found a statistically significant difference between the treated and the control group only for the first six months of the observation time The late effect of dietary prophylactic measures in the present study, as compared to the very early and transient effect of anticoagulant treatment, indicates a difference in the mode of action.

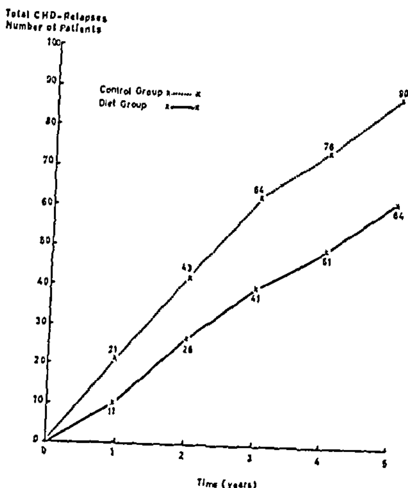


Figure 13 Cumulated incidence of total CHD relapses

3 Serum cholesterol level

A more detailed analysis of this subject has been presented in Figs 11-23 and in Table 27

The serum cholesterol values of the relapse patients refer to the values prior to the relapse Thus the number of CHD relapse patients for whom cholesterol values have been given decreases during the observation period, as is evident from Tables 1 and 5 and from Figs 8-13

It appears from Figs 14 and 15 that there is a difference, but more pronounced in the diet group than in the control group, between the serum cholesterol level in patients with and without CHD relapses In the diet group the serum cholesterol reduction was somewhat greater in the relapse free than in those with CHD relapses In the control group, although the curves partly overlap, the mean value for the whole period of observation was lower in the relapse-free than in those with relapses

When the material is split into two age groups, as is done in Table 27, it appears that the difference mentioned above occurred only in the patients below the age of 60, in whom it was pronounced

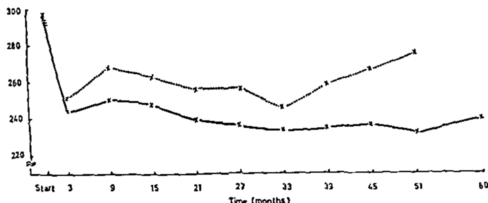


Figure 14. Diet group Mean serum cholesterol level during the observation time in dieters with and without CHD relapses.

	N	Age	Mean cholesterol (mg per 100 ml)		
			Start	Whole obs time (start value incl.)	Reduction (per cent)
CHD +	64	57 5	296	263	14.2
CHD −	142	55 7	297	246	19 2

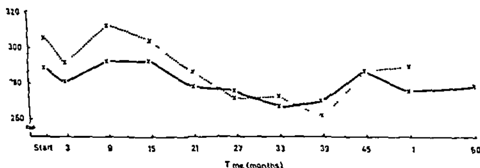


Figure 15 Control group Mean serum cholesterol level during the observation time in controls with and without CHD relapses.

	N	Age	Mean cholesterol (mg per 100 ml)		
			Start	Whole obs time (start value incl.)	Reduction (per cent)
CHD +	90	56 4	306	296	4.2
CHD −	116	56 6	289	277	4.2

Table 27 *Mean serum cholesterol value (mg per 100 ml) during the observation time in patients with and without CHD relapses*

	Diet group				Control group			
	Age < 60		Age ≥ 60		Age < 60		Age ≥ 60	
	N	Mean cholesterol	N	Mean cholesterol	N	Mean cholesterol	N	Mean cholesterol
Myocardial reinfarction	17	279	17	250	27	314	27	279
Acquired angina pectoris	6	274	2	242	12	314	8	287
Sudden death	10	262	12	257	8	290	8	280
Total CHD relapses	33	273	31	252	47	310	43	280
Without CHD relapses	101	246	41	248	76	281	40	281

When the two age groups were analyzed with regard to the CHD relapse rate, as in Figs 16 and 17, the following features emerge At the age below 60 (Fig 16) the CHD relapse rate in the control group was only slightly higher than in the diet group when the initial serum cholesterol value was below 275 mg per 100 ml However, with an initial serum cholesterol value ≥ 275, the CHD relapse rate per 1000 patients was 278 in the diet group against 469 in the control group, a statistically significant difference ($\chi^2 = 5\ 92$, $p = \ 015$)

As expected the CHD relapse rate was higher in the older age group (Fig 17) A high initial serum cholesterol value was not associated with a high relapse rate, and the difference between the diet group and the control group was less

In the following comparisons (Figs 18-23) the purpose has been to study the correlation between the serum cholesterol level and the CHD relapses The data from the two groups have therefore been combined. From Figs 18 and 19, in which the CHD relapse rate has been correlated to the course of the mean cholesterol level, it again appears that below the age of 60 the relapse rate was correlated to the serum cholesterol level, but not in those ≥ 60 years of age

When the various types of CHD relapses are viewed against the serum cholesterol level (Fig 20) it appears that in the sudden death patients the average serum cholesterol level was nearly as low as in those without any relapse In those ≥ 60 years no such difference as that seen in Fig 20 was found, and the data are not given However, the mean serum cholesterol values for the various types of CHD relapses have been given for both age groups in Table 27

The same features presented in a slightly different manner appear in Fig 21 Sudden death is not related to the cholesterol reduction achieved, whereas the total CHD relapses are,

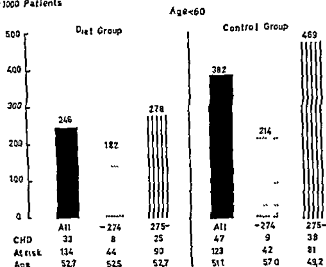


Figure 16 Age < 60 CHD relapse rate in relation to the serum cholesterol level at start.

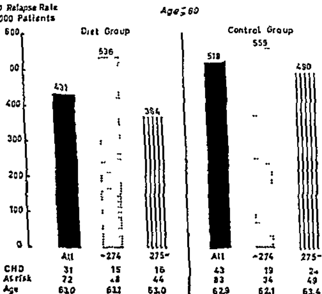


Figure 17 Age ≥ 60 CHD relapse rate in relation to serum cholesterol level at start.

Table 27 *Mean serum cholesterol value (mg per 100 ml) during the observation time in patients with and without CHD relapses*

	Diet group				Control group			
	Age < 60		Age ≥ 60		Age < 60		Age ≥ 60	
	N	Mean cholesterol	N	Mean cholesterol	N	Mean cholesterol	N	Mean cholesterol
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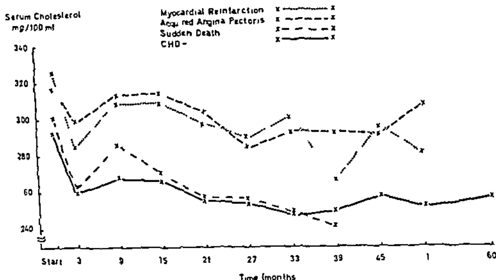


Figure 20 Both groups together Age < 60 Mean serum cholesterol levels during the observation time in patients with different types of CHD relapses and in the relapse-free

	N	Age	Mean cholesterol (mg/100 ml)	
			Start	*Whole obs time* (start value incl.)
Myocard. reinfarct.	44	50 5	326	301
Acquired ang pect.	18	50.2	317	301
Sudden death	18	55 1	301	274
CHD –	177	52 7	293	261

but to a much more pronounced ex tent in those below the age of 60

Finally the material has been ana lyzed in the manner shown in Figs 22 and 23 The contrast between the two age groups is now striking There is a steep increase in the CHD re-lapse rate with increasing serum cho-lesterol levels in those younger than 60 the incidence being about 10 times as high in the highest as in the lowest serum cholesterol group In the pa-tients ≥ 60 years of age only small differences occurred.

It is established that the serum cholesterol and the CHD relapse rate were considerably reduced in the diet group as compared with the control group It is also demonstrated that those patients in the diet group who remained relapse-free had on an aver-age a larger reduction of their se-rum cholesterol than the other pa-tients in the diet group

In an analysis of the CHD relapse rate as related to the initial serum cholesterol value and the mean value in the course of the study, it was

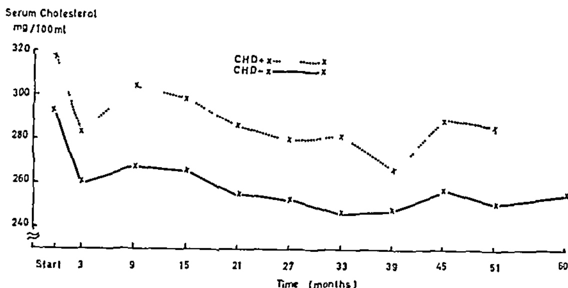


Figure 18 Both groups together Age < 60 Mean serum cholesterol level during the observation time in patients with and without CHD relapses

	N	Age	Mean cholesterol (mg/100 ml)	
			Start	Whole obs time (start value incl.)
CHD +	80	51 3	318	295
CHD −	117	52 7	293	261

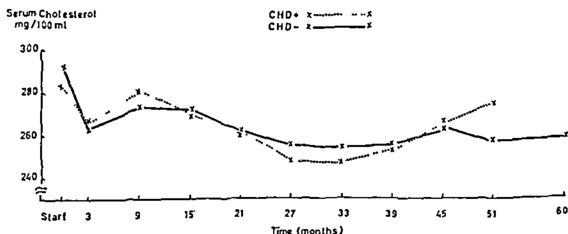


Figure 19 Both groups together Age ≥ 60 Mean serum cholesterol levels during the observation time in patients with and without CHD relapses

	N	Age	Mean cholesterol (mg/100 ml)	
			Start	Whole obs time (start value incl.)
CHD +	74	62 8	284	268
CHD −	81	63 1	293	264

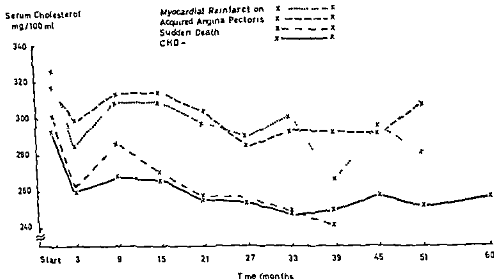


Figure 20 Both groups together Age < 60 Mean serum cholesterol levels during the observation time in patients with different types of CHD relapses, and in the relapse-free

	N	Age	Mean cholesterol (mg/100 ml)	
			Start	Whole obs time (start value incl)
Myocard. reinfarct.	44	50 5	326	301
Acquired ang pect.	18	50 2	317	301
Sudden death	18	55 1	301	274
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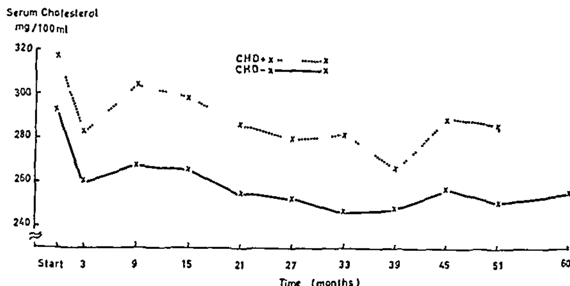


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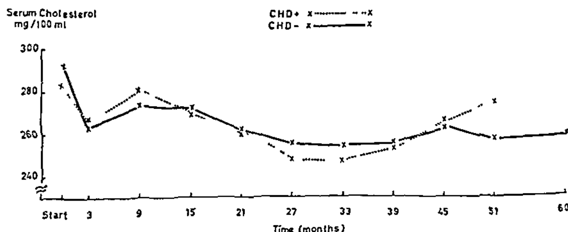


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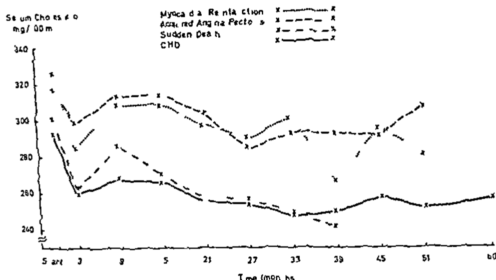


Figure 20 Both groups together Age < 60 Mean serum cholesterol levels during the observation time in patients with different types of CHD relapses and in the relapse-free

	N	Age	Mean cholesterol (mg/100 ml)	
			Start	Whole obs time (start value incl.)
Myocard. reinfarct.	44	50.5	326	301
Acquired ang pect.	18	50.2	317	301
Sudden death	18	55.1	301	274
CHD –	177	52.7	293	261

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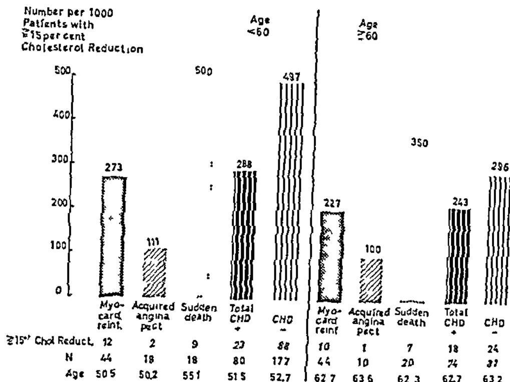


Figure 21 Both groups together Frequency distribution of ≧ 15 per cent cholesterol reduction in patients with and without CHD relapses

found useful to pool the data from the two groups Next, the splitting of the material into two age groups, viz one of patients below the age of 60, and one of those of 60 years and over, yielded some striking results

At the age of 60 years and over, the mean serum cholesterol was at the same level in those with and without CHD relapses in both the diet and the control group

The results achieved in patients below the age of 60 are quite otherwise In fact, the total CHD relapse rate was about 10 times as high in the patients with cholesterol values higher than 300 mg per 100 ml than in those with values below 200

On the other hand, sudden death was found to be unrelated to the serum cholesterol level

It is of interest to compare the statistical data of this study with those of Westlund & Nicolaysen (1966), which also deals with the predictive value of the cholesterol level in Oslo residents In 6,886 healthy males, age 40-59, they found a risk of myocardial infarction, including sudden death, or angina pectoris, 10 times as high at a *serum* cholesterol level above 350 mg per 100 ml as at below 200

Since the effect of the cholesterol-reducing diet with regard to the relapse rate of myocardial infarction and angina pectoris was found to be highly significant, and since there was a strong correlation between the serum cholesterol level and the CHD relapse rate, it is justifiable to conclude that in the present study, a low

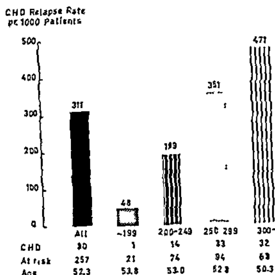


Figure 22 Both groups together Age < 60 Incidence of CHD relapses in relation to mean serum cholesterol level during the observation time.

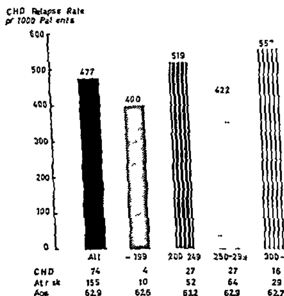


Figure 23 Both groups together Age ≧ 60 Incidence of CHD relapses in relation to mean serum cholesterol level during the observation time.

level of serum cholesterol has been highly beneficial in preventing relapses of CHD in the patients below the age of 60 This was the case irrespective of whether such low levels were maintained through inherent metabolic regulation or were achieved by dietary means

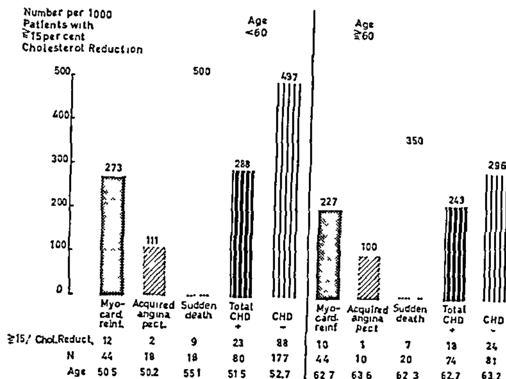


Figure 21 Both groups together Frequency distribution of ≥ 15 per cent cholesterol reduction in patients with and without CHD relapses

found useful to pool the data from the two groups Next, the splitting of the material into two age groups, viz one of patients below the age of 60, and one of those of 60 years and over, yielded some striking results

At the age of 60 years and over, the mean serum cholesterol was at the same level in those with and without CHD relapses in both the diet and the control group

The results achieved in patients below the age of 60 are quite otherwise In fact, the total CHD relapse rate was about 10 times as high in the patients with cholesterol values higher than 300 mg per 100 ml than in those with values below 200

On the other hand, sudden death was found to be unrelated to the serum cholesterol level

It is of interest to compare the statistical data of this study with those of Westlund & Nicolaysen (1966), which also deals with the predictive value of the cholesterol level in Oslo residents In 6,886 healthy males, age 40-59, they found a risk of myocardial infarction, including sudden death, or angina pectoris, 10 times as high at a serum cholesterol level above 350 mg per 100 ml as at below 200

Since the effect of the cholesterol-reducing diet with regard to the relapse rate of myocardial infarction and angina pectoris was found to be highly significant, and since there was a strong correlation between the serum cholesterol level and the CHD relapse rate, it is justifiable to conclude that in the present study, a low

Table 29 *Distribution of myocardial reinfarctions according to anticoagulant therapy*

	Diet group				Control group			
	Total		Fatal		Total		Fatal	
	N	%	N	%	N	%	N	%
On anticoagulant therapy	26	60 5	5	50 0	38	59 4	16	69 6
Not on anticoagulant therapy	17	39.5	5	50 0	26	40 6	7	30 4
Total	43	100	10	100	64	100	23	100

Table 30 *Distribution of acquired angina pectoris according to anticoagulant therapy*

	Diet group		Control group	
	N	%	N	%
On anticoagulant therapy	6	60 0	17	58 6
Not on anticoagulant therapy	4	40 0	12	41.4
Total	10	100	29	100

Table 31 *Distribution of sudden death according to anticoagulant therapy*

	Diet group		Control group	
	N	%	N	%
On anticoagulant therapy	24	88 9	17	63 0
Not on anticoagulant therapy	3	11.1	10	37 0
Total	27	100	27	100

Table 32 *Distribution of total CHD relapses according to anticoagulant therapy*

	Diet group		Control group		Both groups	
	N	%	N	%	N	%
On anticoagulant therapy	56	70 0	72	60 0	128	64 0
Not on anticoagulant therapy	24	30 0	48	40 0	72	36 0
Total	80	100	120	100	200	100

5 Angina pectoris at the start of the trial

Figure 24 presents the CHD relapse rate in the patients who were without angina pectoris at the start of the trial The difference between the groups in favor of the diet group with regard to the rate of reinfarction and acquired angina are highly significant. In spite of the fact that the difference between the groups with regard to sudden death is not significant a highly significantly

Table 28 *Long term anticoagulant treatment during observation time* *

\+ On anticoagulant treatment
\+ % Per cent on anticoagulant treatment
\- Not on anticoagulant treatment

	Diet group			Control group		
	+	+ %	-	+	+ %	-
Start	165	80 1	41	148	71 8	58
3 months	164	80 4	40	147	72 4	56
9 »	156	78 8	42	142	72 4	54
15 »	151	77 0	45	139	72 0	54
21 »	145	75 5	47	130	68 1	61
27 »	140	74.5	48	126	68 1	59
33 »	132	72 1	51	122	70 1	52
39 »	109	60 6	71	105	62.5	63
45 »	95	56 2	74	98	60 9	63
51 »	88	52 7	79	97	61 0	62
60 »	80	50 0	80	82	55 0	67

* All patients regularly controlled are listed. The reluctant patients and the cancer patient who died suddenly are also included.

4 Long-term anticoagulant therapy

The anticoagulant drugs used were dicoumarol and phenylindandione, with about the same frequency At start of the trial and throughout the whole observation period, the patients were examined and the results carefully recorded with regard to the use of anticoagulants Thus, I had full information about any changes in such treatment In cases of doubt, the physician in charge of the treatment was contacted In the cases of CHD relapses, additional information was obtained through the hospital records

In general, there were only small changes in the individual patients with regard to start and withdrawal, the changes mainly occurring in connection with hospitalization for CHD relapses, and thus, as a rule, after the CHD relapse

More detailed information on anticoagulant treatment has been given in Tables 28-32 It appears that in the two groups anticoagulant treatment was about equally frequent This also applies when the various types of CHD relapses are grouped and the data studied

In the present study anticoagulant treatment was not especially randomized, and valid conclusions as to the effect of this therapy in preventing CHD relapses cannot be drawn On the other hand, it is of importance to note that anticoagulant treatment was equally frequent in the two groups during the trial, the mean percentage on anticoagulant treatment in the diet group and the control group being 69 7 and 67 3 respectively Further, the percentages of patients on anticoagulant treatment who suffered CHD relapses do not indicate that one group benefited more than the other by anticoagulant therapy

Table 33 *Blood pressure in patients with and without CHD relapses*

		Diet group		Control group		Both groups	
		CHD +	CHD −	CHD +	CHD −	CHD +	CHD −
Mean BP at start	Systolic	163.2	157.1	156 8	151 5	159 4	154.5
	Diastolic	99 0	96 0	95 4	92 0	96 9	94.3

6 Blood pressure

Data on the blood pressure are given in Figs 26-29 and in Tables 33 and 34.

The mean blood pressure remained unchanged during the trial (Fig 26) The relative distribution of normotensives and hypertensives (Table 34) changed very little during the observation period.

From Table 33 it appears that the mean blood pressure at the start of the trial was slightly higher in the patients of both groups who experienced CHD relapses during the observation period.

A more detailed analysis of the prognostic importance of the blood pressure has been given in Figs 27 and 28 In the diet group the CHD relapse rate was only slightly higher in the hypertensives than in the normotensives However in the control group the CHD relapse rate was markedly higher in the hypertensives as compared with the normotensives

The higher cholesterol level in the controls is the most probable explanation of the different significance of an elevated blood pressure in the two groups This contention is supported by the data presented in Fig 29 In both groups the CHD relapse rate was increased in the hypertensives with an elevated initial serum cholesterol level ($\geq$ 275 mg per 100 ml) as compared with the normotensives with the same initial cholesterol level However, this was especially pronounced in the control group, in which the CHD relapse rate per 1,000 patients was 408 in the normotensives as compared with 652 in the hypertensives This difference is nearly significant on the five per cent level ($x^2 = 3.22$, p = 073)

Thus, it seems justified to state that the unfavorable influence of an elevated blood pressure on the CHD relapse rate was of greater importance in the control group than in the diet group As the blood pressure remained unchanged during the trial, it is reasonable to conclude that the reduction of the plasma cholesterol level yielded protection to the dieters against the deleterious influence of a high blood pressure

7 ECG findings

The distribution of patients with a normal and a pathological ECG at the start has been given previously (Table 2 III M) In Figs 30 and 31 the CHD relapses are presented in patients with a normal and with a pathologic ECG at the start of the

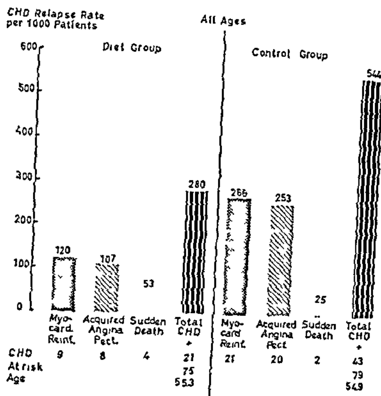


Figure 24 CHD relapse rate in patients without angina pectoris at the start of the trial

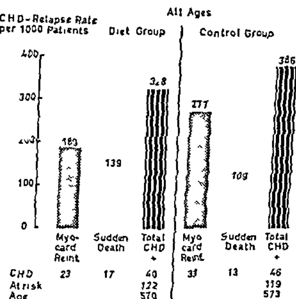


Figure 25 CHD relapse rate in patients with angina pectoris at the start of the trial

lower total CHD relapse rate was found in the diet group ($x^2 = 10.00$, $p = .002$)

In the patients who had angina pectoris at the start, there was also a higher relapse rate in the control group (Fig 25) However, this difference is not statistically significant on the five per cent level

Thus, the reduction of cholesterol was of greater importance in those who were without angina pectoris at the start of the trial

It also appears that the incidence of sudden death in both groups taken together was greater in the patients with angina pectoris than in those without ($x^2 = 7.30$, $p = .007$)

Table 33 *Blood pressure in patients with and without CHD relapses*

		Diet group		Control group		Both groups	
		CHD +	CHD –	CHD +	CHD –	CHD +	CHD –
Mean BP at start	Systolic	163.2	157.1	156 8	151.5	159 4	154.5
	Diastolic	99 0	96 0	95 4	92 0	96 9	94.3

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The mean blood pressure remained unchanged during the trial (Fig 26) The relative distribution of normotensives and hypertensives (Table 34) changed very little during the observation period.

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Thus, it seems justified to state that the unfavorable influence of an elevated blood pressure on the CHD relapse rate was of greater importance in the control group than in the diet group As the blood pressure remained unchanged during the trial, it is reasonable to conclude that the reduction of the plasma cholesterol level yielded protection to the dieters against the deleterious influence of a high blood pressure

7 ECG findings

The distribution of patients with a normal and a pathological ECG at the start has been given previously (Table 2 III M) In Figs 30 and 31 the CHD relapses are presented in patients with a normal and with a pathologic ECG at the start of the

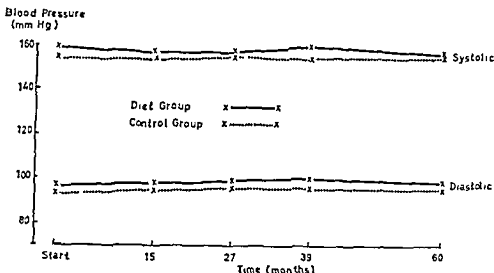


Figure 26 Blood pressure during the observation time

	Mean value
Diet	158 2/98 6
Control	154 3/95 5

Table 34 *Frequency distribution of normotensives and hypertensives throughout the observation time*

Diet group

Blood pressure		Start N	Start Per cent	Start Mean BP	27 months N	27 months Per cent	27 months Mean BP	60 months N	60 months Per cent	60 months Mean BP
Systolic	-160	127	61 1	142	125	67 5	145	94	61 8	143
	165-	79	38 9	188	60	32 5	183	58	38 2	180
Diastolic	-90	84	40 8	84	58	31.3	86	71	46 7	86
	95 100	56	27 2	98	66	35 6	99	40	26 3	98
	105-	66	32 0	113	61	33 1	113	41	27 0	112

Control group

Blood pressure		Start N	Start Per cent	Start Mean BP	27 months N	27 months Per cent	27 months Mean BP	60 months N	60 months Per cent	60 months Mean BP
Systolic	-160	154	74 6	141	131	71 6	144	104	70 3	142
	165-	52	25 4	191	52	28 4	185	44	29 7	185
Diastolic	-90	110	53 4	84	89	48 6	86	82	55 4	85
	95 100	59	28 6	98	56	30 6	99	35	23 6	99
	105-	37	18 0	116	38	20 8	114	31	21 0	112

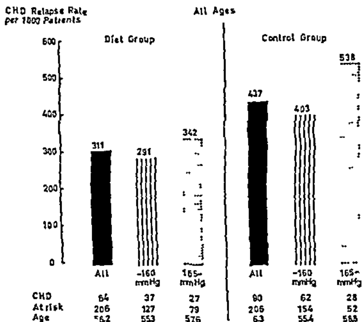


Figure 27 CHD relapse rate in relation to systolic blood pressure at the start of the trial

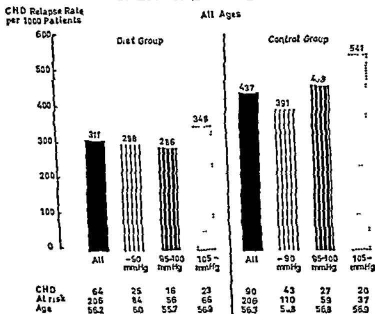


Figure 28. CHD relapse rate in relation to diastolic blood pressure at the start of the trial.

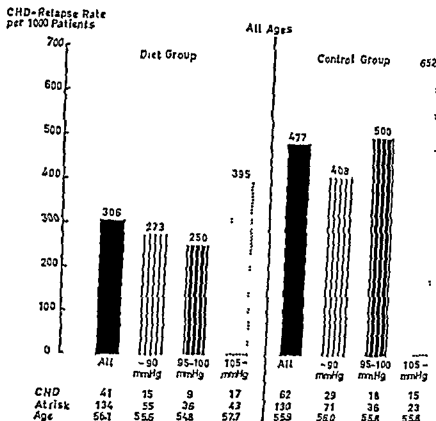


Figure 29 CHD relapse rate in relation to diastolic blood pressure at start in patients with a start serum cholesterol ≥ 275 mg per 100 ml

trial It appears that the difference in the CHD relapse rate between the groups was approximately the same whether the ECG was normal or not. Further, it is demonstrated that the total CHD relapse rate in both groups was higher in the patients with a pathological ECG at the start of the trial This was especially true with regard to the incidence of sudden death In both groups together only 3 per cent of the patients with a normal ECG at start suffered sudden death as compared with 13 5 per cent in those with a pathologic ECG This difference is highly significant (χ^2 = 11 80, p = 001)

Thus the plasma cholesterol level was of the same importance in patients with *previous myocardial infarction* whether the ECG had been normalized or not The relapse rate, however, was *greater when the ECG* was pathologic This was especially true with regard to sudden death

8 Relative heart volume

Fig 32 presents the total CHD relapse rate in the patients in the diet and the control group with a heart volume below and above 500 ml per m² body surface at the time of the primary myocardial infarction

In the patients with a relative heart volume less than 500 ml, the total CHD relapse rate per 1,000 patients was 258 and 458 in the diet group and the control group respec-

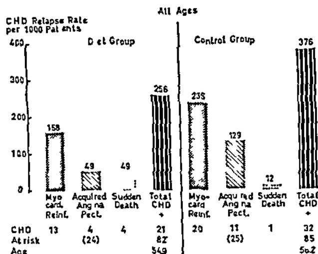


Figure 30 CHD relapse rate in patients with a normal ECG at the start of the trial. In parentheses number at risk of acquiring angina pectoris.

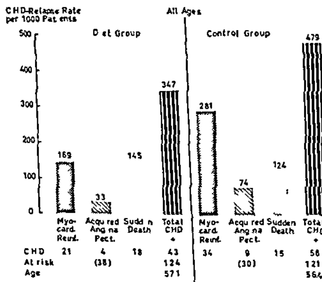


Figure 31 CHD relapse rate in patients with a pathological ECG at the start of the trial

In parentheses number at risk of acquiring angina pectoris

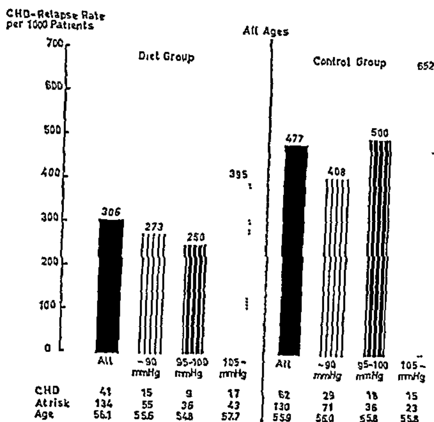


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Fig 32 presents the total CHD relapse rate in the patients in the diet and the control group with a heart volume below and above 500 ml per m² body surface at the time of the primary myocardial infarction

In the patients with a relative heart volume less than 500 ml, the total CHD relapse rate per 1,000 patients was 258 and 458 in the diet group and the control group respec-

Table 35 *The relation between height and weight at the start and the end of observation (last examination)*

	Diet group		Control group	
	Start	End of observation	Start	End of observation
Underweight > 20 %	0	1	1	1
» 16-20 %	6	7	4	5
» 11 15 %	7	16	5	14
Normal weight	124	120	113	104
Overweight 11 15 %	19	20	37	30
» 16-20 %	16	20	17	20
» 21 25 %	22	10	9	15
» 26-30 %	5	5	13	6
» 31 35 %	3	4	5	4
» 36-40 %	2	2	0	4
» > 40 %	2	1	2	3
Total	206	206	206	206

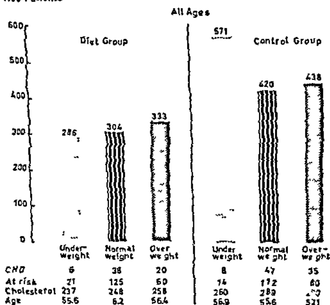


Figure 34 CHD relapse rate in the diet and the control group in relation to height/weight.

Height value at start.
Weight mean value during observation time.
Cholesterol mean value during observation time

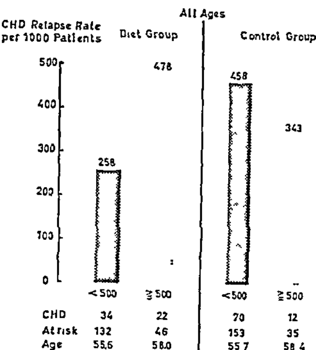


Figure 32 CHD relapse rate in relation to relative heart volume

tively The difference is statistically significant (x^2 = 11 38, p = 001)

However, in the patients with a relative heart volume above 500 ml, there was no significant difference between the groups (x^2 = 0 99, p = 32)

Thus, the plasma cholesterol-reducing diet proved to be beneficial only in patients with a normal heart size

9 *Body weight*

Data on body weight have been presented in Table 35 and in Figs 33-36 It appears that the mean body weight in the control group changed very little during the trial (Fig 33) In the diet group a mean weight reduction of about 2 5 kg took place during the first 3 months of the trial From

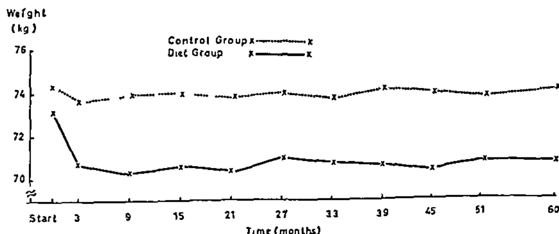


Figure 33 Mean weight in the diet and the control group during the observation time

	N (at start)	Start value (kg)	Mean reduction (kg)	Mean reduction (per cent)
Control	206	74 3	0 5	0 7
Diet	206	73 2	2 5	3 4

Table 35 *The relation between height and weight at the start and the end of observation (last examination)*

	Diet group		Control group	
	Start	End of observation	Start	End of observation
Underweight > 20 %	0	1	1	1
» 16-20 %	6	7	4	5
» 11 15 %	7	16	5	14
Normal weight	124	120	113	104
Overweight 11 15 %	19	20	37	30
» 16-20 %	16	20	17	20
» 21 25 %	22	10	9	15
» 26-30 %	5	5	13	6
» 31 35 %	3	4	5	4
» 36-40 %	2	2	0	4
» > 40 %	2	1	2	3
Total	206	206	206	206

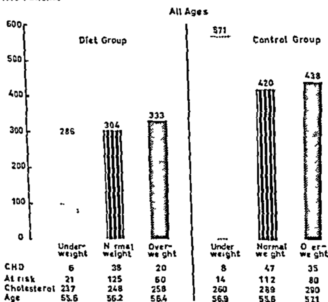


Figure 34. CHD relapse rate in the diet and the control group in relation to height/weight.

Height value at start.
Weight mean value during observation time.
Cholesterol mean value during observation time

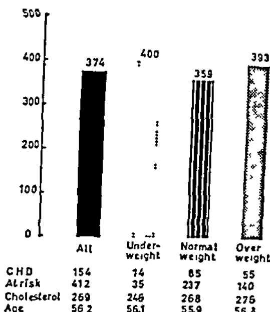


Figure 35 CHD relapse rate in both groups together in relation to height/weight.

then on, the mean body weight also remained unchanged in the dieters

The distribution of normal weight, underweight, and overweight also changed little during the observation period (Table 35) At the end of the observation, 11 in the diet group and 10 in the control group had become underweight Overweight had disappeared in 7 dieters and in one control

It appears from Figs 34 and 35 that there was a slight trend towards a higher serum cholesterol level with increasing weight, as shown in larger groups by Berge & Nicolaysen (1963) In the present study these differences in serum cholesterol level were almost entirely between the underweight and normal weight patients In the normal weight and overweight controls the mean serum cholesterol level was the same, and in the dieters those overweight had only slightly higher serum cholesterol level than those with normal weight.

In neither of the groups was there any significant difference in the CHD relapse rate as related to body weight (Fig 34), with the exception of the underweight controls, in whom the CHD relapse rate was somewhat higher

The reason the underweight dieters had the same and the controls a higher CHD relapse rate, in spite of a lower serum cholesterol level, might be that they were thin because they were more affected by their atherosclerotic disease, as the higher relapse rate in the underweight controls may indicate, in exhausted persons serum cholesterol tends to fall Thus Selvaag (1962) demonstrated a lower serum cholesterol level in patients with severe symptoms of peripheral obliterative atherosclerosis than in those without such symptoms

The greater weight reduction in the diet group appeared at the first examination, 3 months after the start of the trial, but from then on, body weight did not change This weight reduction was most probably induced by the change of diet. As no general recommendation of reduction of calories had been given to any groups, the weight reduction must be considered as a side effect of the recommended diet.

Serum cholesterol level tends to fall during periods of weight reduc-

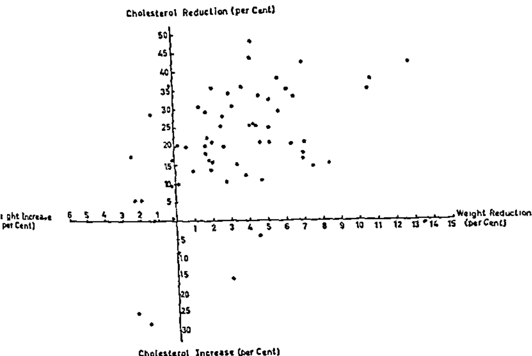


Figure 36 Diet group Change of serum cholesterol correlated to change of weight.

tion, as shown by Walker et al (1953), and recently by Galbraith et al. (1966) who by means of a 900-calorie diet induced a considerable loss of weight in profoundly obese adults, accompanied by a considerable reduction of the serum cholesterol as well as the serum triglycerides, in spite of a high cholesterol intake.

The greater weight reduction in the diet group may therefore to some extent be responsible for the reduced serum cholesterol level. However, this may only be true to a limited degree. After the initial fall in the serum cholesterol to 241 mg per 100 ml at 3 months, there was a rise to 254 at 9 months, and from then on a fall to 233 at 33 months The mean body weight, however, did not change during this period.

Further, as seen in Fig 36, serum cholesterol was reduced in 34 dieters (17 per cent) when their weight was unchanged or increased, and in 15 dieters (7 5 per cent) serum cholesterol increased in spite of the fact that body weight remained unchanged or was reduced. Moreover, the mean weight reduction of 123 dieters with a ≥ 15 per cent cholesterol reduction was approximately the same as in the whole diet group (3 6 as against 3 4 per cent)

Finally the mean weight reduction of the dieters with CHD relapses was the same (3.2 per cent) as that

of the relapse-free (3 5 per cent), while the serum cholesterol was less reduced (14 2 per cent against 19 2 per cent)

However, there is a positive correlation in the present study between the reduction of body weight and the reduction of serum cholesterol, the coefficient of correlation being +0 24 (p < 01)

In conclusion, the greater weight reduction in the diet group was unintended There is a correlation between the reduction of serum cholesterol and the reduction of body weight. However, there was no definite relation between the CHD relapse rate and the height/weight Nor was there any definite difference in the mean serum cholesterol level in those of normal weight as compared with the overweight Finally, the CHD relapse patients in the diet group had approximately the same weight reduction as the relapse-free Their serum cholesterol reduction, however, was less

10 Smoking habits

As shown in Table 36 smoking habits did not change significantly during the trial in either of the groups

The CHD relapse rate in the control group (Fig 37) was approxi-

Table 36 *Distribution of smoking habits at the start and the end (last examination) of the observation time*

	Diet group		Control group	
Smoking habits (definitions p 15)	Start	End	Start	End
None	70	71	76	78
Light	49	47	49	47
Moderate	72	79	63	62
Heavy	15	9	18	19
Total	206	206	206	206

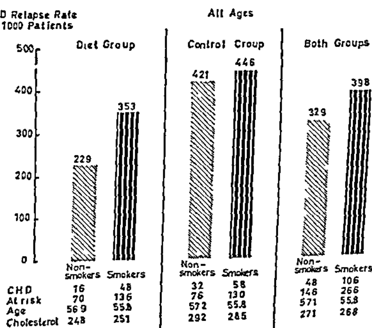


Figure 37 CHD relapse rate in relation to smoking habits.

mately the same in smokers and in non smokers In the diet group the relapse rate was somewhat higher in the smokers than in the non smokers However, the difference is not statistically significant on the five per cent level (x^2 = 2 78, p = 096)

The relation between age, mean serum cholesterol level, and smoking habits is also presented in Fig 37, demonstrating no significant differences

Sudden death was not related to smoking habits, the actual rates being 12.3 per cent in non smokers and 13.5 per cent in smokers

11 Combined risk factors

In order to evaluate the predictive importance of the serum cholesterol level in the presence of other known coronary risk factors, the CHD relapse rate was studied in three different combinations, two of which included serum cholesterol For this purpose data from the two groups were pooled (Fig 38)

In the first combination, low serum cholesterol and normal diastolic blood pressure versus high serum cholesterol and elevated diastolic blood pressure, the total CHD relapse rates were 184 and 444 per 1,000 patients respectively The difference is statistically significant (x^2 = 8 05, p = 005)

In the second combination, low serum cholesterol in non smokers ver sus high serum cholesterol in tobacco smokers, the total CHD relapse rates were 118 and 435 per 1,000 patients, this difference also being highly significant (x^2 = 9 99, p = 002)

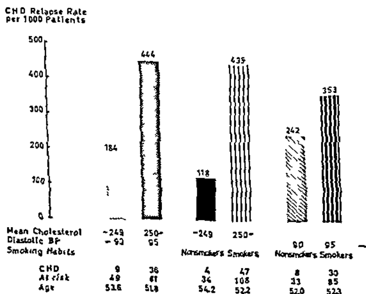


Figure 38. Both groups. Age < 60 CHD relapse rate in relation to combined risk factors.

In the third combination, normal blood pressure in non-smokers versus high blood pressure in smokers, there was a trend in favor of the non-smoking normotensives The difference, however, is not statistically significant.

Thus it is demonstrated that in the combinations of risk factors in which the serum cholesterol was included there was a highly significant increase in the CHD relapse rate, in contrast to the combination excluding the serum cholesterol level

12 Absence of risk factors

Figure 39 presents the incidence of CHD relapses in the patients of the two groups who at the time of their primary myocardial infarction had a relative heart volume less than 500 ml, and who at the start of the trial had a diastolic blood pressure ≤ 90 mm Hg, and were without angina pectoris

These qualifications were present in 21 dieters and 32 controls, and their mean serum cholesterol value at the start of the trial was 286 and 301 mg per 100 ml respectively

In these dieters, myocardial reinfarction and sudden death did not occur, while 3 of them acquired angina pectoris In contrast, 9 of the controls had a reinfarction, 10 acquired angina pectoris, and 1 died suddenly The figures for total CHD

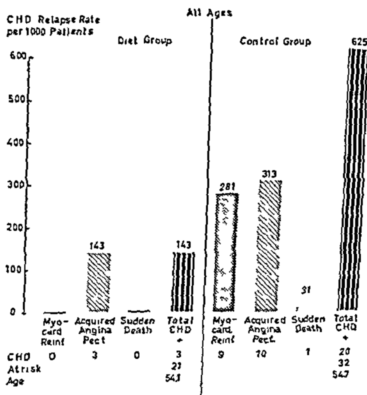


Figure 39 CHD relapse rate in patients with a normal diastolic blood pressure, a normal relative heart volume, and without angina pectoris at the start of the trial

relapses per 1,000 are 143 in the diet group against 625 in the control group The difference between the groups is highly significant (x^2 = 10.15, p = 001)

In these persons, who had overcome their previous myocardial infarction with no lasting signs of clinical heart disease, thus supposedly constituting the subgroups most comparable to the normal male population at the same age level, the cholesterol reducing diet had its most favorable effect.

To summarize, the serum cholesterol value proved to be the only one of the above factors which had a great influence on the CHD relapse rate The unfavorable effect of a high serum cholesterol level was especially pronounced when combined with an elevated blood pressure and with tobacco smoking

The greatest beneficial effect of the plasma cholesterol reducing diet was encountered in those persons who at the start of the trial had a normal diastolic blood pressure, a normal relative heart volume, and did not have angina pectoris

Chapter 8

GENERAL DISCUSSION

The choice of adequate groups of patients is of decisive importance for a controlled therapeutical trial

In a trial with postmyocardial infarction patients, the patients could be randomized during their primary infarction, or at a later time

The patients drawn for the diet group would possibly more readily accept a change of diet when randomized early than at a later stage It might also have been an advantage that the examiner became acquainted with all the patients during their stay in hospital

However, the drawbacks to this approach were considered to be greater than those to the other one The collection of a suitable material would have required considerable time It was also thought to be an advantage to avoid the greater incidence of coronary relapses in the first year after a myocardial infarction which dietary measures might not be expected to influence Finally, the plasma cholesterol reduction following an acute myocardial infarction might be a disturbing factor in later comparisons In consequence, it was decided that at least one year should have elapsed since the primary infarction

The length of the observation time was another important question If a reduction of the plasma cholesterol level should prove to be beneficial, it seemed probable that some time would be needed before it became effective On the other hand, survivors of a myocardial infarction are relatively advanced in age and may have intercurrent diseases which could interfere

It is generally accepted that, unless a controlled study is followed by sequential analysis, the length of the observation period should be decided before the start of the trial It was therefore decided that the trial should last for exactly 5 years

The groups of the trial had to be sufficiently large to permit valid conclusions even after division into subgroups, but not so large that the quality of the follow-up study would suffer even when performed by a single person The size of the present material proved to be suitable for the purpose

In the present material the diagnosis of the primary myocardial infarction was not verified by means of uniform diagnostic criteria However, autopsy results indicate a high degree of diagnostic accuracy In 47 autopsies in the present study, an

old myocardial infarct was found in 46 In one case a dissecting aortic aneurysm was found and no definite myocardial infarct (Table 6)

The material is representative of a certain geographic area, which should be an advantage in generalizing from the results

Of crucial importance for a controlled trial is the strict comparability of the test groups, and shortcomings in this respect were found to be an important objection to most of the previous controlled dietary trials

In the present study the groups were established by a strictly randomized system With regard to all measurements in which a personal bias can be excluded, such as age and the serum cholesterol level, there was no difference between the groups The conclusion that the groups at the start of the trial were equally at risk of having relapses of their coronary heart disease seems justified. However, if the slightly higher blood pressure in the diet group was of importance, it adds to the risk of the dieters

The success of the present trial depended entirely upon the degree of diet adherence in the diet group and upon the unchanged diet in the control group during the observation time. As previously stated both these factors were satisfactory, the meta bolic proof being the results of the serum cholesterol determinations A highly significant difference between the groups was achieved. The initial serum cholesterol values were the same for the two groups During the observation period the mean cholesterol level in the diet group was about 14 per cent below that of the control group

Samples for serum cholesterol determinations were drawn independently of meals, as previous observations have demonstrated the diurnal variations in serum cholesterol to be irregular (Leren 1960) Moreover, the cholesterol determinations were performed at the same time of the day and the year in both groups, so that diurnal or seasonal variations were eliminated as sources of error in comparison of the groups

The observed incidence of total CHD relapses was significantly lower in the diet group Analysis of the material reveals that this held true only for reinfarction, fatal and nonfatal, and for new cases of angina pectoris The incidence of sudden death, however, was the same in the two groups

Splitting of the material into age subgroups revealed that the lower incidence in the diet group of total CHD relapses, like that of myocardial infarction and acquired angina pectoris, was statistically significant only in the younger age groups At the age of 60 and over no statistically significant reduction of CHD relapses could be demonstrated.

The reduction of the serum cholesterol level associated with a reduced CHD relapse rate strongly suggests a cause and effect relationship This contention receives additional support by the findings of a definitely

Chapter 8

GENERAL DISCUSSION

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In the present material the diagnosis of the primary myocardial infarction was not verified by means of uniform diagnostic criteria However, autopsy results indicate a high degree of diagnostic accuracy In 47 autopsies in the present study, an

Elderly people, however, having survived a myocardial infarction, may according to this study be exempted from strict dietary restrictions, as factors other than the plasma cholesterol level seem to be the decisive ones

A more optimistic view of the possibility of a primary prevention of coronary heart disease by means of diet finds support in the finding that the most striking effect induced by plasma cholesterol reduction was achieved in those who had recovered from their primary infarction with a normal diastolic blood pressure, a normal heart size, and without angina pectoris These subgroups are thought to represent those most comparable to the general male population at the same age level

A brief discussion of the mecha nism through which the disease may be influenced by the level of choles terol in the plasma is warranted.

The major lipid component of the atherosclerotic artery is cholesterol in free and esterified form Bottcher et al. (1960) reported that of the lipids extracted from the atherosclerotic intima of coronary arteries, triglycerides represented nearly 50 per cent in the early stages and 27 per cent in the more advanced stages However, Insull & Bartsch (1966) analyzed fatty streaks and fibrous plaques from aorta for their lipid composition At the same time the adjacent normal intima was also analyzed. When the values for the normal intima were subtracted from the values for the atherosclerotic lesions it appeared that triglycerides were eliminated Of the remaining lipids about 90 per cent was cholesterol and only 10 per cent phospholipids In view of these observations new analyses of the composition of the atherosclerotic intima of coronary arteries are needed before it can be accepted that triglycerides accumulate in the course of the development of atherosclerosis in these arteries

Chemical analyses by Giertsen (1964) of the aorta, coronary arteries, and cerebral arteries of 417 individuals showed that the cholesterol/phospholipid ratio was an objective and valid chemical index of the degree of atherosclerosis, as judged by gross examination

Animal experiments have demonstrated that cholesterol is transferred to the arterial wall roughly in proportion to the serum cholesterol concentration Observations in familiar hypercholesterolemia indicate that this also holds good in man It is therefore possible that in advanced atherosclerosis a reduction of the plasma cholesterol concentration will also reduce the rate of transfer, and thus reduce the risk of clinical events occurring

Thrombus formation in the coronary arteries is a frequent occurrence and it may be that a reduction of plasma cholesterol or a change in the composition of the plasma lipids can influence this process as well However in the present material there are no data which can usefully be discussed in this connection.

lower serum cholesterol level in the CHD relapse-free than in those with relapses This distinction applies only to cases with reinfarction and new cases of angina pectoris below the age of 60 Significant difference in relapses between the diet group and the control group was also limited to those below 60 years of age

The preventive value of plasma cholesterol reduction is further indicated by the finding that the difference between the groups in the incidence of CHD relapses was more pronounced when the serum cholesterol level at the start was highly elevated.

It was also demonstrated that the plasma cholesterol-reducing diet to some extent reduced the deleterious effect in coronary heart disease of an elevated blood pressure

The fact that statistical significance was achieved only after the lapse of some time favors the concept of a dietary effect on CHD relapses and of the nutritional-metabolic concept of atherosclerosis Thus, the difference in the incidence of total CHD relapses first became significant in the third year of the trial This is in contrast to the reported effect of anticoagulant therapy in postmyocardial infarction patients, in which the favorable effect was limited to the first six months after the infarction (Bjerkelund 1957)

This difference also indicates a difference in the mode of action Anticoagulant therapy exerts its effect on blood coagulation, and its main effect in coronary heart disease may be in the prevention of the thrombo-embolic complications of atherosclerosis The late effect of dietary measures as presented in this study suggests a causal effect on atherosclerosis per se

The incidence of sudden death was not influenced by the diet, nor did the serum cholesterol level of those who died suddenly differ significantly from that of the relapse-free This disappointing observation was by no means surprising Although associated with coronary atherosclerosis, sudden death in survivors of myocardial infarction may not be directly related to the degree of atherosclerosis, but perhaps more to localization. Sudden death is most frequently caused by ventricular fibrillation or heart stand-still, probably released by ischemia in vital parts such as the impulse-conducting system The absence of influence of the plasma cholesterol level on sudden death is therefore not irreconcilable with the contention that the prescribed diet has an anti-atherogenic effect

It has been maintained that the plasma cholesterol level is of little prognostic value for the life expectancy of survivors of a myocardial infarction (Little et al 1965) In the present study this holds true only for the total CHD relapses in patients of 60 years of age and above, and for sudden death at all ages

However, it is quite important for the prevention of CHD relapses that in the younger age groups dietary measures proved to be effective even after a myocardial infarction

and fat from other sources (2 6 per cent) Of the mean dietary fat, 21 6 per cent was saturated, 25 7 monounsaturated, and 52 7 polyunsaturated.

The mean reduction of serum cholesterol in these 17 dieters was 31 *per cent.*

Details of the way of instruction and training, which were found to be essential, are given The adherence to the diet was controlled by close personal contact assisted by a full time, experienced dietician working in the homes of the patients The degree of adherence could be quantified by means of a detailed questionnaire used 6 times during the observation period.

Of the dieters, 62 2 per cent were judged to be 'excellent' adherers, 22 1 per cent 'good', 10 3 per cent fair', and 3 9 per cent 'bad'

Judging by personal contact with the controls and by a questionnaire at the end of the trial, and also by the serum cholesterol level, which remained nearly unchanged from the beginning to the end, it can be assumed that the type of food in the control group remained largely unchanged.

At the start of the trial the mean serum cholesterol value was exactly the same in the two groups (296 mg per 100 ml) From the first control examination, 3 months later, and throughout, a significant difference in the serum cholesterol level persisted. The mean cholesterol reduction in the diet group was 17 6 per cent against 3 7 per cent in the control group Of the dieters 123 obtained a serum cholesterol reduction of $\geq$ 15 per cent, against 27 of the controls

In the diet group 43 myocardial reinfarctions occurred in 34 patients Ten of the reinfarctions were fatal. In the control group, 64 myocardial reinfarctions occurred in 54 patients Twenty-three of the reinfarctions were fatal

The difference between the groups is statistically significant (reinfarction patients x^2 = 5.22, p = 022, fatal reinfarctions x^2 = 4 74, p = 029) The difference did not become statistically significant until the third year of the trial

Of 75 dieters and 79 controls who did not have angina pectoris at the start of the trial, 10 dieters and 29 controls acquired angina during the observation period. Of these 2 of the dieters and 9 of the controls also had reinfarctions, and have been counted as such After excluding these patients the difference between the groups is still statistically significant (x^2 = 4 61, p = 031)

The incidence of sudden death as defined on p 20 was the same in the two groups, 27 in each. This result was not surprising Sudden death is considered in most instances to be caused by ischemia, provoking electrical instability followed by ventricular fibrillation or heart stand still. It is reasonable therefore, that sudden death *in survivors* of a myocardial infarction presumably with rather advanced atherosclerosis, is less related to the degree of atherosclerosis than to other factors, such

Chapter 9

SUMMARY

The purpose of the present study was to evaluate what effect a reduction of the plasma cholesterol concentration by means of diet would have on morbidity and mortality from coronary heart disease (CHD relapses) in male survivors of myocardial infarction

A pilot study which preceded the present trial demonstrated that a sizeable plasma cholesterol reduction could be achieved by the planned experimental diet, and valuable experiences were gained

The present material consisted of 412 males aged 30-64, and discharged from medical departments in Oslo during the years 1956-58, with a first diagnosis of myocardial infarction They were allocated at random to the experimental diet group and to the control group, 1-2 years after their infarction

At the start of the trial it was decided that the trial should last for exactly 5 years

The comparability of the groups was thoroughly studied, and it was found that they were strictly comparable except for blood pressure However, blood pressure was slightly higher in the diet group

Only one CHD relapse was counted in each patient, the order of priority being myocardial reinfarction, new cases of angina pectoris, and sudden death. Criteria for the diagnosis of reinfarction were established, and the decision was left to a diagnostic board which did not know to which group the patient belonged

A close follow-up study was undertaken, including clinical examination with diet and weight control, serum cholesterol determination and electrocardiography, and a review of hospital records

The cholesterol-lowering diet was low in animal fats and in dietary cholesterol, and rich in vegetable oil

In an analysis of the experimental diet as consumed by 17 selected dieters, the mean daily intake was 92 g protein, 104 g fat, 269 g carbohydrates, and 264 mg cholesterol Daily intake of calories was 2,387

Fat calories covered 39 per cent of total calories The sources of fat were soy bean oil (72 per cent), fish fat (11 6 per cent), animal fat (8 8 per cent), cereal fat (5 0 per cent),

those with angina pectoris at the start of the trial the difference in the CHD relapse rate between dieters and controls was not statistically significant

Significantly reduced relapse rate in the dieters as compared with the controls was found only in those with a normal relative heart volume at the time of the primary infarction

The presence of a pathological ECG at the start increased the CHD relapse rate in both groups, especially the incidence of sudden death, which in both groups together was significantly higher when the ECG was pathological (x^2 = 11 80, p = 001) However, the reduction of the CHD relapse rate in the dieters was the same, whether the ECG at the start was normal or not.

Investigation of the relation of CHD relapse rate to the coronary risk factors which can be influenced by preventive measures, viz serum cholesterol, blood pressure, and tobacco smoking, revealed that the serum cholesterol level was the factor which had by far the strongest influence on the CHD relapse rate

When the groups were analyzed together for this purpose it appeared that in the normotensives with a low serum cholesterol level and in the non smokers with a low cholesterol level, the CHD relapse rate was significantly reduced as compared to the hypertensives with a high cholesterol and to the smokers with a high cholesterol (Fig 38) The respective tests of significance are x^2 = 8 05, p = 005, and x^2 = 9 99, p = 002

The correlation between the serum cholesterol level and the CHD relapse rate at age below 60 (both groups together) was striking (Fig 22) The data are as follows a mean serum cholesterol value during the trial < 200, 200-249, 250-299, and ≥ 300 (mg per 100 ml) was associated with a total CHD relapse rate per 1,000 patients of 48 189 351, and 471 respectively

In the patients who had overcome their previous myocardial infarction with no obvious signs and symptoms of heart disease, that is without angina pectoris and with a normal heart volume and a normal blood pressure a highly significant difference between the diet group and the control group in the total CHD relapse rate was found (x^2 = 10 15, p = 001)

Thus in these subjects who are believed to constitute the subgroups of the material most comparable with the general male population at the same age level, dietary reduction of plasma cholesterol proved to be most effective in the prevention of CHD relapses

as the localization of the lesions to more vulnerable areas

The total number of CHD relapses was 80 in the diet group and 120 in the control group These relapses occurred in 64 dieters and in 90 controls The difference is statistically significant (x^2 = 6 48, p = 011), and the difference became significant during the second year of the trial

Subdividing the material into age groups, those below 60 years and those of 60 years and above, revealed that the difference in the CHD relapse rate was statistically significant only in the patients below the age of 60 So, too, was the relapse combination, myocardial infarction and acquired angina pectoris The incidence of sudden death was the same in the two groups, independent of age

In the diet group as well as in the control group, the serum cholesterol level was higher in the patients with CHD relapses than in those without Subdivision of the material revealed that this difference in the serum cholesterol level was also limited to those under 60 years of age who had myocardial reinfarctions and acquired angina pectoris At the age of 60 and above, there was no difference in the serum cholesterol level between those with and without CHD relapses

No association was found between the serum cholesterol level and the incidence of sudden death in either of the two age groups

The CHD relapses were also related to other possible coronary risk factors

Body weight had no definite influence on the serum cholesterol level or on the CHD relapse rate, except that the cholesterol level tended to be reduced in those underweight

Blood pressure had no definite influence on the CHD relapse rate in the diet group In the control group there was a strong trend, though statistically not significant, toward a higher CHD relapse rate in the hypertensives This was especially true when serum cholesterol at the start was ≥ 275 mg per 100 ml The difference in CHD relapses between these normotensive and hypertensive controls escapes the five per cent level of significance very narrowly (x^2 = 3 22, p = 073)

Smoking habits did not influence the serum cholesterol level or the CHD relapse rate in the control group In the smoking dieters there was a suggestion, although statistically nonsignificant, toward a higher CHD relapse rate (x^2 = 2 78, p = 096)

The presence of angina pectoris at the start of the trial increased the CHD relapse rate in both groups, especially the incidence of sudden death, which in both groups together was significantly higher in those with angina than in those without (x^2 = 7 30, p = 007)

The effect of cholesterol reduction was most pronounced in those who were without angina pectoris at the start of the trial The difference in the CHD relapse rate between these dieters and controls was highly significant (x^2 = 10 00, p = 002) In

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CONCLUSION

The effect of a plasma cholesterol-lowering diet on the CHD relapse rate was studied for 5 years in a group of 206 male infarction patients, and the results compared with those in another group of 206 patients who continued on a conventional diet.

The patients were allocated to the groups after a strictly randomized system, and the groups matched well

The cholesterol-lowering diet reduced the incidence of total CHD relapses

There was a distinct difference in the effect on patients *below* the age of 60 and those of 60 and over The difference was highly significant in the younger age group, but not in the older

Sudden death occurred with an identical rate in the diet group and the control group, so that the significance applies to myocardial reinfarction — fatal and non-fatal — and to new cases of angina pectoris, but not to sudden death

The most striking effect of dietary reduction of the plasma cholesterol was encountered in those who had recovered from their previous myocardial infarction with no obvious signs and symptoms of their heart disease *These subjects are thought* to be most comparable to the general population

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SUPPLEMENTUM 468

OBSTRUCTIVE ARTERITIS OF TAKAYASU'S TYPE

CLINICAL ROENTGENOLOGICAL AND LABORATORY STUDIES ON 36 PATIENTS

by

JOUKO A PALOHEIMO

HELSINKI 1967

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CONTENTS

INTRODUCTION

An obstructive arteritis of the aorta and its branches in young patients especially in women, has been accorded much interest in recent years Ophthalmological changes of this disease were presented by the Japanese ophthalmologist Takayasu 1908 (46) according to whom it has been called Takayasu s Disease» and »Takayasu s Arteritis (9 24)

Cases of this disease have been published mostly from the Orient, but in the past few years in increasing numbers from the Western countries (3 4 7—11 24 25 29 31 37 40 41 43, 44) The disease has many names for example pulseless disease brachial arteritis young female arteritis syndrome of occlusion of the supra aortic trunks obliterative brachiocephalic arteritis and reversed coarctation It is one of the causes of aortic arch syndrome (38) Names such as subisthmic coarctation elongate coarctation stenosing aortitis middle aortic syndrome and central aortitis point to the more systemic nature of the disease

According to Strachan (43) the disease can be divided into two phases The *early systemic* or pre-pulseless phase shows systemic manifestations such as fatigue fever night sweats, anaemia, cough, pleurisy, pleural effusion, haemoptysis, pericarditis, locomotor manifestations, ulceration or erythema nodosum type lesions of the legs, abnormalities of the plasma proteins, persistent elevation of the erythrocyte sedimentation rate, and inflammatory changes in the arterial walls Changes in the pulmonary artery have been seen (15,24) and rarely venous involvement has been encountered (20 40) In the *late occlusive* or pulseless phase the systemic symptoms are more or less lacking stenosis of the aorta itself or of orifices of the major arteries arising from the aorta are the causes of »pulselessness of upper and lower extremities. Ischaemic symptoms of brain trophic changes of the head and extremities intermittent claudication, effort angina cardiac involvement and renal artery stenosis with or without hypertension are seen in this phase

The etiology of this aorta -arteritis is unknown. The most widely held opinion is that the disease *is autoimmune* in origin (24 40 43) Although circulating antibodies against human artery (20, 44) or heart (23) could not be demonstrated symptoms of

mined by a modification of the method of Hultman (22). The *tuberculin test* was made with two tuberculine: a purified protein derivative and Alttuberculin. The hydroxyproline (HOP) level of serum and its excretion into the urine were determined by the method of Prockop and Udenfriend (36) with some modifications (26).

Biopsies were taken from the temporal artery, skin and muscle, and in some cases specimens were obtained during operation.

Control studies have been made at intervalls of 3 months to 1—2 years.

METHODS

In taking the *history*, inquiry was made of the occurrence of rheumatic diseases in the family, smoking habits, child bearing, a list of viral and bacterial diseases, with special attention to streptococcal infections, rheumatic fever, rheumatoid arthritis, and other joint symptoms, and symptoms of cardiovascular and renal diseases were noted *Clinical examination* was made with special attentions to the arteries and the heart The state of the thyroid gland, liver and spleen and of the skin muscles and joints was examined Blood pressure measurements were made from the arms and legs, and from the aorta during aortography Eye ground examination was supplemented with ophthalmodynamometry in many cases Electrocardiography was performed with chest leads V_{1-6} X-rays of the chest, lumbar spine and sacroiliac joints were taken and arterial calcifications of the abdominal aorta and femoral arteries were looked for *Angiographic studies* were made by the retrograde method of Seldinger

Laboratory results are presented in Tables 7 and 8 The laboratory studies covered the blood picture, electrolytes (K, Na, Ca, P) of serum, protein analysis, enzyme activity (GOT, LDH, alk phosphatase), serum cholesterol, creatinine, protein bound iodine (PSJ), iron and the iron binding capacity (TIBC), Wassermann reaction (cholesterol Wassermann, Kahn, sitolipin), antistreptolysin titre (AST), antistaphylolysin titre (ASta), Waaler-Rose test, latex fixation, DNA- antibodies, thyroid antibodies (antithyroglobulin and antimicrosomal antibodies), LE cell phenomenon, blood group (ABO, Rh), Coombs reactions coagulation factors (Quick, Factor V, Factor VII, Stuart factor prothrombin, P + P, recalcification time, thromboplastin generation test) and fibrinogen level Immunoelectrophoresis of serum was made using agar gel diffusion Vitamin A absorption was tested giving 3000 I U of vitamin A per os after 12 hours' fasting and making the measurements before and and four hours after the test (5,35) In the intravenous glucose tolerance test 100 ml of 25 per cent glucose solution was given, and in the peroral tolerance test 1 gram of glucose was given per kg of body weight Blood glucose was deter-

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RESULTS

AGE AND SEX

The ratio of women to men in this series was 23 to 13, or about 1 8 to 1 The age and sex distribution of the series at the time of this examination and at the onset of symptoms are presented in Table 1

In the youngest age groups there were more women (15 of the 18 patients under 40 years were women) but in the older groups the ratio was about one to one The time between the probable onset of the disease and the beginning of symptoms of arterial changes varied from 0 to 12 years

Table 1 THE AGE AND SEX OF 36 PATIENTS WITH OBSTRUCTIVE ARTERITIS OF TAKAYASU S TYPE AT THE TIME OF THIS EXAMINATION AND AT THE ONSET OF SYMPTOMS

Age group	At this examination		At onset of symptoms	
	female	male	female	male
15—20	2	0	9	0
21—25	5	0	3	1
26—30	2	2	2	3
31—35	3	0	2	1
36—40	3	1	5	3
41—45	1	3	3	2
46—50	4	5	0	3
50—	3	2	0	0
Total	23	13	23	13

HISTORY

Family history In 5 instances, or in 14 per cent of the series there were cases of rheumatoid arthritis in the family the brother of Case 5 had a severe juvenile rheumatoid arthritis, the father of Case 21 had had severe rheumatoid arthritis for 10 years before acute death from heart disease at age 49 Both grandmothers and two brothers of Case 23, father and mother of Case 31, and the mother of Case 35 had rheumatoid arthritis The mothers of Cases 1 and 34, and the brother of Case 26 had bronchial asthma Myocardial infarction had occurred in close family members of six patients

Smoking habits There were 18 cigarette smokers or 50 per cent of all patients Over 20 cigarettes were smoked daily by 12 patients, five of whom were women, from 10 to 20 cigarettes daily by 6 patients, including two women The smokers were from

Table 2 THE PREVIOUS AND CONCOMITANT DISEASES OF 36 PATIENTS WITH OBSTRUCTIVE ARTERITIS OF TAKAYASU'S TYPE

Disease	Case No	Total	Per cent of total
Recurrent tonsillitis	4 5 8 9 11 12, 13 14 17 18 21 27 32	13	36
Rheumatic fever	8 11, 17 20	5	14
Rheumatoid arthritis	—	0	0
Other joint symptoms	8 9 10 12 15 19 23 27 30 35	10	28
Ankylosing spondylitis	3 4, 5 14	4	11
Systemic lupus erythematosus	28	1	3
Myasthenic syndrome	34	1	3
Myocardial infarction	12 16 32	3	8
Nontuberculous lung disease	5	1	3
Tuberculosis active	25	1	3
Tuberculosis, inactive	7	1	3
Chronic pyelonephritis	4 22 26	3	8
Cholelithiasis	11 13 16	3	8

33 to 63 years of age the older patients smoked more than the younger

Child bearing and its effect on symptoms Fourteen patients had given birth to one or more children 10 of them after onset of the disease All the infants were normal The symptoms of arterial disease were mild during pregnancy but in 7 of these 10 patients the symptoms became exacerbated in connection with or some weeks after the delivery

Previous and concomitant diseases In Table 2 are presented the previous and concomitant diseases of the patients

In 13 of the 36 patients there was recurrent tonsillitis in the history, and in most of these cases tonsillectomy had been done Rheumatic fever had occurred in four of them. Typical cases of rheumatoid arthritis were not seen, but in one (Case 8 with recurrent bursitis) the biopsy diagnosis of the joint capsule was »changes typical to rheumatoid arthritis and Case 23, also with recurrent bursitis, had been treated at a rheumatism hospital as a case of atypical rheumatoid arthritis 14 years prior this study Four patients had x ray changes typical to ankylosing spondylitis One of them had had ankylosing spondylitis 3 years before symptoms of arterial disease (Case 14) two others had had back symptoms (Case 3 and 4), and one (Case 5) was symptomless.

SYMPTOMATOLOGY

The symptoms of the patients at the time of this study are listed in Table 3 The first symptoms are also presented

It was seen that general malaise joint symptoms and symptoms of arterial ischaemia of the upper and lower extremities and of the brain were the most usual. Case 2 had no subjective

RESULTS

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Table 3 THE SYMPTOMS OF 36 PATIENTS WITH OBSTRUCTIVE ARTERITIS OF TAKAYASU S TYPE AT THE TIME OF THIS STUDY, AND THE FIRST SYMPTOMS

Symptom	No of cases	% of total	First symptom	% of total
Fever	4	11	2	6
General malaise	16	45	8	22
Anaemia	4	11	2	6
Rheumatic fever	4	11	2	6
Joint symptoms	10	28	3	8
Pain in neck	5	14	3	8
on swallowing	2	6	1	3
in upper chest	7	19	2	6
in abdomen	2	6	0	0
in shoulder, arm	9	25	4	11
Raynaud's phenomenon	4	11	3	8
Claudication of				
upper extremity	19	53	10	28
lower extremity	10	28	5	14
Angina pectoris	10	28	5	14
Myocardial infarction	3	8	1	3
Hypertension	9	25	1	3
Tachycardia	4	11	1	3
Dyspnoea	9	25	0	0
Pericarditis	2	8	1	3
Cerebral symptoms				
vertigo dizziness	15	42	2	6
visual disturbances	9	25	3	8
headache	11	30	3	8
hemiplegia	4	11	1	3
Thirst	1	3	0	0
Hoarse voice	1	3	0	0
Muscular atrophy	2	6	0	0
Muscular weakness	1	3	1	3

symptoms other than poor vision in the left eye The pain in the neck was sometimes a very intensive ache, and in some cases a tenderness of the carotid arteries was noted In two of these patients there was pain on swallowing, especially when drinking cold beer At first the neck pains had been diagnosed in all cases as lymphangitis colli with pharyngeal infection. The patient with muscular weakness (myasthenic syndrome) had had bronchial asthma since the age of 10

RESULTS OF CLINICAL EXAMINATION AND ANGIOGRAPHY

Signs of arterial disease At palpation the signs of arterial disease were a hard consistency of the artery, abnormal pulsation or pulselessness, and a thrill Auscultation revealed a murmur over the stenosed arteries, sometimes only as a short »shot» but in most cases as a long harsh murmur

Angiography showed stenosis and post-stenotic dilatation, defects in filling, and in four cases a reversed blood flow through the vertebral artery into the subclavian artery (subclavian steal)

The multiplicity of arterial changes was striking in most of the cases The results of angiography are presented in Table 4 and Figures (see Appendix)

Table 4 AFFECTED ARTERIES IN 36 PATIENTS WITH OBSTRUCTIVE ARTERITIS OF TAKAYASU'S TYPE.

Artery	No of cases	% of total
Aorta ascendens	10	28
descendens	3	8
abdominalis	16	45
Truncus brachiocephalicus dx	10	28
Arteria carotis dx	11	31
sin	15	42
Arteria subclavia dx	20	55
sin.	26	72
Arteria vertebralis dx	4	11
sin.	6	17
Arteria mesenterica sup	1	3
Arteria renalis dx.	8	22
sin.	5	14
Arteria iliaca dx and sin.	17	47
Arteria femoralis	8	22
Arteria pulmonalis	1	3
The syndrome of subclavian steal	4	11

The most commonly affected artery was the left subclavian The subclavian steal occurred in 4 cases, all of them on the left side in Case 22 a bilateral subclavian steal was possible One or more of the arteries arising from the aortic arch were affected in all cases and the abdominal aorta with iliac arteries and or renal arteries was also involved in most cases. In two cases an axillary artery and in one case the proximal one third of femoral artery were locally stenosed

Arterial calcifications were seen in 7 patiens in 5 in the abdominal aorta and in three in the femoral and/or iliac arteries The aortic arch was calcified more than normally in 6 cases

Signs of heart disease Table 5 pressents the history and clinical findings of heart disease

It was seen that 24 of the 36 patients had some symptom or sign of heart disease Three of them had only a slight systolic ejection sound in the aortic area In 6 patients an aortic diastolic murmur was heard in two of them this began during the follow-up period. None had, however, haemodynamically significant aortic valvular disease The P—Q interval was lengthened in 2 patients, LBBB was seen in one and PRBBB in one Two patients had a myocardial infarction in their history and one during the treatment with persisting ECG changes The picture of left ventricular hypertrophy was seen in 7 patients. The heart size was greater than normal (500 cc/sq.m) in 10 patients in one of whom there was moderate pericardial effusion (patient with SLE) Of the patients with an enlarged heart size 3 had hypertension

Blood pressure and its correlation to angiographic changes in renal arteries None of the patients had malignant hypertension, but a large pulse pressure with slightly elevated values was seen in 8 patients (Table 6) Among the 25 patients examined by renal arteriography there were 9 who had renal arterial stenosis, 4 of them bilaterally Six of these 9 had hypertension and 3 were normotensive Two of the pa-

Table 5 SYMPTOMS AND SIGNS OF HEART DISEASE IN 36 PATIENTS WITH OBSTRUCTIVE ARTERITIS OF TAKAYASUS TYPE.

Case No	Anginoid pain in history	Myocardial infarction in history	ECG	Enlarged heart size cm^3 per sq m.	Murmur of AS	Murmur of AI
1	—	—	slight LVH	—	+	—
2	—	—	—	—	+	—
4	—	—	—	—	+	+
5	—	—	P-Q 0 24 s	505	++	—
6	—	—	slight LVH	575	+	
9	—	—	pericarditis	—	+	—
10	—	—	notched QRS	—	+	++
11	+	—	tachycardia LVH	555—810	+	+
12	+	+	ant infarction, LBBB	625	—	—
14	—	—	PRBBB	500	—	—
16	+	+	inf infarction	—	+	—
18	+	—	slight LVH	—	+	—
19	—	—	—	—	++	—
20	+	—	LVH, ventr extrasyst	—	+	—
21	+	—	notched QRS	540	—	—
22	—	—	—	—	+	—
24	—	—	LVH	535	+	—
26	+	—	slight LVH	—	++	—
27	+	—	T negat. III, aVF	535	+	—
28	—	—	slight LVH	over 1000 (effusion)	+	—
29	+	—	P-Q 0.22 s	620	—	—
30	—	—	tachycardia	—	+	+
32	+	—	slight LVH, post infarction	—	+	+
36	—	—	—	—	+	+

AS = aortic stenosis, AI = aortic insufficiency LVH = left ventricular hypertrophy, LBBB = left bundle branch block PRBBB = partial right bundle branch block.

tients with hypertension had renal artery stenosis and more than one renal artery to one or both kidneys Of the 16 patients withouth renal artery stenosis two had hypertension and one was the patient with SLE One normotensive patient had two renal arteries on the right side

RESULTS OF LABORATORY STUDIES

Most of the results of the laboratory studies are presented in Table 8 (Appendix) Summaries of the results are made below

The blood group was determined in 35 cases, and the results are seen in Table 7

One of the patients of B Rh- Group had a rare Dasher blood group The distribution of the blood groups differed somewhat from that of the normal Finnish population (A 41 9 per cent, O 32 5 per cent, B 18 2 per cent, AB 7 4 per cent, Rh + 85per cent, Rh-

Table 6 ARTERIAL BLOOD PRESSURE AND RENAL ARTERY PATHOLOGY ACCORDING TO ANGIOGRAPHIC FINDINGS IN 36 PATIENTS WITH OBSTRUCTIVE ARTERITIS OF TAKAYASU'S TYPE

	Blood pressure				Renal artery stenosis		
Case No	right arm	left arm	leg	aorta during angiography	right	left	Multiple renal arteries
1	145/80	0/0	160/95	120/80	—	—	2 right
2	155/80	90/70			arteriography not done		—
3	125/75	125/75			—	—	—
4	135/60	80/70	135/70		a.g	not done	
5	0/0	0/0	200/100		a.g	not done	
6	150/80	120/90			—	—	—
7	0/0	0/0	170/105		a.g	not done	
8	190/130	170/120			—	—	—
9	170/100	120/100	250/140	(170/80 *post operat.*)	+	+	—
10	0/0	0/0	105/55		—	—	—
11	125/95	200/105	250/115	220/85	+	—	—
12	130/80	80/65	105/?		a g	not done	
13	110/75	80/0			a.g.	not done	
14	0/0	0/0	240/140		+	+	3 left
15	135/90	115/80	120/95	135/93	—	—	—
16	135/110	105/100	150/85		—	—	—
17	110/100	140/90			—	—	—
18	205/100	205/100			+	+	—
19	130/75	115/65		135/65	—	—	—
20	90/75	90/75	130/?		—	—	—
21	110/90	110/90	190/90		+	+	—
22	130/70		160/85		—	—	—
23	115/85	100/85			a.g.	not done	
24	160/95	120/90		180/90	—	+	—
25	160/100	150/90	165/130		+	—	2 left 2 right
26	140/80	110/85			—	—	—
27	150/100	120/90	165/120		a.g	not done	
28	190/110	155/90			a.g	not done	
29	125/80	0/0	130/75		—	—	—
30	180/110	0/0	145/75		a.g	not done	
31	140/80	130/75	120/75		—	—	—
32	170/110	105/85			+	—	—
33	110/?	110/?	180/120		+	—	—
34	120/70	120/70	140/90		—	—	—
35	140/120	180/120	230/160		a g	not done	
36	120/50	75/55	130/60		—	—	—

Table 7 BLOOD GROUP OF 35 PATIENTS WITH OBSTRUCTIVE ARTERITIS OF TAKAYASU'S TYPE

Blood group	No. of patients	% of total	Blood group	No of patients	% of total
A Rh+	16	51.5	O Rh+	5	20
A Rh—	2		O Rh—	2	
B Rh+	5	20	AB Rh+	3	8.5
B Rh—	2		AB Rh—	0	
			Rh+		83
			Rh—		17

15 per cent) in the predominance of group A, but no further conclusions could be made because of the smallness of this series

Erythrocyte sedimentation rate was from 2 to 20 mm per first hour in 10 patients, or in 28 per cent, from 20 to 40 in 7 patients, or 20 per cent, and from 41 to 126 in 19 patients, or 52 per cent The highest values were seen in patients with the most acute clinical picture The group with high ESR, from 41 to 126, comprised 14 women and 5 men

The haemoglobin level was or had been low (under 11 g per 100 cc of blood) in 12 patients, all women

The serum iron level was low in most of the cases, with an elevated or normal total iron binding capacity The etiology of this iron deficiency was unknown in most cases In one patient a low iron absorption was seen, and some gave a history of gynaecological bleedings

The *leukocyte count* was under 10 000/cc^3 of blood, except for 8 patients, or 19 per cent of cases, who had counts of 10 500 to 15 700 withouth any reasonable explanation In 3 cases this finding was only transient Relative *eosinophilia* was from 5 5 to 9 in 5 patients, or in 15 per cent of 33 cases studied

Thrombocytes over 350 000 per cc^3 were seen in 11 patients of 33 These patients had thrombocytosis of 350 000 to 704 000 transiently or permanently, and severe disease pictures with multiple arterial stenoses

The *serum cholesterol level* was over 300 mg per cent in 10 patients, or in 30 per cent, in three of them from 350 to 399 mg per cent, 23 patients or 64 per cent of cases, had a value of 200 to 299, and under 200 mg per cent was seen in 3 patients The three patients with the highest cholesterol values were all women, aged 45, 45 and 51 They had the arterial disease in a chronic state, with calcifications of the abdominal aorta Their clinical picture was more »arteriosclerotic» than that of others Two of them had a pathologic glucose tolerance curve

Serum protein bound iodine (PBI) was studied in 24 patients, the values being from 4 7 to 8 7 gamma per cent withouth hypo- or hyperthyrosis in I^{131} studies

Serum uric acid level was normal, under 7 mg per cent, in all of the 25 patients studied

SGOT and SLDH determinations were made routinely The SGOT level in these patients was low, 17 of 32 being from 8 to 14 Wroblewski units and the others from 15 to 30 SLDH levels were normal, from 200 to 500, and the isoenzyme distribution, examined only in a few cases, was normal

Serum proteins and paper electrophoresis The total serum protein level was between 6 5 and 8 0 g per cent in 34 of 36 patients Case 25 had values of 8 7 and 8 2 g per cent, Case 13 5 8 g per cent In 14 cases, or 39 per cent of cases, an elevated gamma-globulin level (from 20 to 32 per cent of total protein) was seen

The serum fibrinogen level was determined in 35 patients and in 22 or 63 per cent of cases, it was over

400 mg per cent, being from 420 to 880 mg per cent The ESR was elevated in all the cases with a high fibrinogen level

Blood coagulation factors determined were normal in 31 of 33 cases In one patient the euglobulin lysis time was prolonged and in one a lengthened recalcification time was observed, caused by an unknown systemic anticoagulant.

Serological tests for syphilis (WaR, cholWaR Kahn, sitolipin) were negative in all cases

Antistreptolysin titre (AST) was over 200 in 9 cases, or 25 per cent, once or more times during the study In Case 8 the AST value varied from 2000 to 280 correlating with the patient s joint and bursitis symptoms

Antistaphylolysin titre (ASta) was elevated only in Case 8 (from 4 5 to 2 5 U) the same case with the highest AST value

Waaler Rose test was or had been positive (from 32 to 240) in 5 cases or 14 per cent of the series In 4 of these the *latex* fixation test was positive At the time of this examination both tests were positive in 3 patients one of whom was the patient with SLE. The other two had advanced arterial changes but only transient joint symptoms and muscular aching

The *LE cell phenomenon* was positive in three patients or 8 3 per cent of the series Case 14 was a male patient with active ankylosing spondylitis Case 22 a female with long standing active arteritis and Case 28 had SLE

DNA antibody test was negative in all cases, also in Case 28 with active SLE.

Thyroid antibodies in serum (antithyroglobulin and antimicrosome antibodies) were studied in all cases. Only Case 20 showed a very weak antithyroglobulin antibody titre of 1 250

Coombs test was made in order to detect the possibility of immunohaemolytic anaemia being one etiological cause of the hypochromic anaemia with high iron binding capacity in these patients The tests were negative except in Case 11, a woman with severe active disease, whose direct and indirect Coombs tests gave a weak positive reaction. This patient had had profuse gynaecological bleedings. Signs of haemolysis (bilirubinaemia, haemoglobinaemia or -uria, and low haptoglobin level of serum) were not detected

Immunoelectrophoretic analysis showed elevated immunoglobins in 18 cases, but no specific picture was seen. The results of immunoelectrophoresis correlated well with the increased level of gamma globulins seen in paper electrophoresis

Hydroxyproline level of serum and hydroxyproline excretion into urine The results of this study were correlated with the normal values of Laitinen et al. (26) The results are seen in Table 8 (in Appendix) The hydroxyproline (HOP) level of serum and the excretion into the urine were at normal levels in most cases. Four patients had a high HOP excretion or/and serum level In 3 of them the disease was in an active phase and

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DISCUSSION

The patients were taken into the present series according to the clinical criteria It is therefore possible that they may have arterial diseases of different etiologies but with the same clinical manifestations The series will be discussed as one group

In acute forms of the disease with systemic symptoms its classification as Takayasu's arteritis is not difficult. However this disease is thought to have an acute (pre-pulseless) phase and a chronic (pulseless) phase (42) In the chronic state it may be very difficult or even impossible to differentiate it clinically from athero sclerosis The symptomatology of obstructive arteritis is very rich. It has been said to be one of the great imitators of medicine (42) In the present series too symptoms from many organs were encountered, there were systemic symptoms such as fever general malaise and anaemia and joint symptoms Inflammation of the arteries was the cause of pain and tenderness in the neck (e g. during swallowing) pain in the upper chest the abdomen and the shoulder Claudication of arms and legs was common and abdominal angina was suspected in one case Heart disease was seen in many cases as effort angina, myocardial infarction, heart murmurs and electrocardiographic changes. Acute pericarditis was present in one patient, and tachycardia in some cases Neurological symptoms such as vertigo dizziness temporary or static visual disturbances diplopia headache temporary or static paralysis were seen, as well as muscular atrophy and weakness. In some patients hypertension was diagnosed

Possibility of an autoimmune nature of the disease

The etiology of Takayasu s arteritis is unknown but the current opinion is that it is one of the autoimmune diseases The onset of obstructive arteritis of Takayasu's type in this series occurred during or after the puberty but in many cases later, or the time of onset could not be determined. In men it seemed to be more frequently insidious without systemic symptoms. In many cases in this series the patients had had recurrent streptococcal infection before the occurrence of arteritis. In two cases tonsillitis had been followed by general malaise and

in one it was chronic No specific conclusions could be drawn

Glucose tolerance test and vitamin A absorption test In 22 cases an intravenous and in 2 cases a peroral glucose tolerance test was made In 11 cases, or 46 per cent of the series, the tolerance curve was pathologic, although none of the patients had a manifest diabetes Two of these 11 patients were under 30 years, one of them 19 years old In two cases the peroral tolerance curve was very low but the intravenous curve was normal

Vitamin A absorption was studied in 17 patients In all of them the result was normal, including Case 26 suspected to having abdominal angina

MANTOUX SKIN TEST

The Mantoux skin test for tuberculosis was made on 21 patients No increased tuberculin sensitivity was seen in patients in this series

BIOPSIES

Biopsies were taken from 29 patients A temporal artery biopsy was taken on 23 occasion Arteritic changes were not seen in the temporal artery biopsies with the exception of some infiltration of round cells in some cases In two cases a skin biopsy was diagnosed as an inactive skleroderma, and in two cases a muscle biopsy showed degenerative changes of collagenosis The biopsy of joint capsule of Case 8 was typical of rheumatoid arthritis

The biopsies also gave no diagnostic findings, although in some cases collagenous or rheumatoid changes were detected The biopsies taken at the operation showed thrombotic changes, thickened intima and in some cases a slight inflammatory reaction

DISCUSSION

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In acute forms of the disease with systemic symptoms its classification as Takayasu s arteritis is not difficult However this disease is thought to have an acute (pre-pulseless) phase and a chronic (pulseless) phase (42) In the chronic state it may be very difficult or even impossible to differentiate it clinically from atherosclerosis. The symptomatology of obstructive arteritis is very rich. It has been said to be one of the great imitators of medicine (42) In the present series too symptoms from many organs were encountered there were systemic symptoms such as fever general malaise and anaemia and joint symptoms Inflammation of the arteries was the cause of pain and tenderness in the neck (e g during swallowing) pain in the upper chest the abdomen and the shoulder Claudication of arms and legs was common and abdominal angina was suspected in one case Heart disease was seen in many cases, as effort angina, myocardial infarction, heart murmurs and electrocardiographic changes. Acute pericarditis was present in one patient, and tachycardia in some cases Neurological symptoms such as vertigo, dizziness, temporary or static visual disturbances, diplopia, headache temporary or static paralysis were seen, as well as muscular atrophy and weakness. In some patients hypertension was diagnosed

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a high ESR, and some months later arterial symptoms appeared In five instances there was a history of rheumatic diseases in the family The most interesting point is that four patients in this series had ankylosing spondylitis, one systemic lupus erythematosus and one myasthenic syndrome as a concomitant disease The literature contains reports of arteritis of the great arteries of Takayasu's type in association with rheumatoid arthritis (14,39), SLE (27), polymyositis (32) and polymyalgia rheumatica (2, 18, 19) The concomitant occurrence of these rare diseases of autoimmune nature cannot be only a coincidence, they should be regarded as manifestations of the same basic disturbance Many patients had had a disease resembling rheumatic fever, but it seems that the etiologies of rheumatic fever and Takayasu's arteritis are different, because no case of Takayasu's arteritis and mitral valvular disease in the same patient has been published In a series from Finland of about 500 patients with mitral stenosis (34) no cases with this obstructive arteritis were seen Autopsies published of patients with Takayasu's disease have revealed no mitral valvular affections

The clinical picture of patients in the acute phase of arteritis was that of autoimmune diseases periodical occurrence of general malaise, fever, high ESR, locomotor symptoms and arterial symptoms There was a predominance of females especially in the younger age group It is known that females are more common victims than males, particularly to SLE and also to rheumatoid arthritis However, this sex incidence is reversed in polyarteritis nodosa and ankylosing spondylitis (28) The sex distribution in various autoimmune diseases is possibly based on a difference in the reactivity of the sexes and on variation and overlapping of the autoimmune process in the body according to »forbidden clones»

A feature common to autoimmune diseases was also seen, i e the effect of pregnancy and delivery on the disease Ten women had had one or more deliveries since the onset of their disease, and in 7 of them the symptoms had became exacerbated in connection with delivery In the case of Sicuranza et al (42) no exacerbation occurred

The multiplicity of arterial changes was striking Earlier it was the general opinion that Takayasu's arteritis would be a disease of the aortic arch only but in recent years the systemic nature has gained more attention Changes have been reported in the whole aorta with all its branches and also in the pulmonary arteries In some instances more peripheral changes have been seen The distal third of the femoral artery and axillary artery were stenosed in the present series, cerebral arterial changes have also been encountered (37) In addition, two cases in the literature (20, 40) had had phlebitis commonly seen in thrombangitis obliterans, but other symptoms of Buergers disease were lacking

In many patients there have been

symptoms and signs of heart disease Coronaritis has been seen at autopsy (40) It may have been the etiology of cardiac infarction in two of our cases as well as of anginoid pains The ECG changes of pericarditis were seen in one patient a prolonged P—Q interval in two and BBB in two Cardiac murmurs without significant valvular disease were heard in 19 patients in 6 of these there was a slight aortic insufficiency Dilatation of the aorta and aortic ring with valvular deformity causes aortic insufficiency, as is also seen in other forms of aortic diseases as in connection with syphilis rheumatoid arthritis and ankylosing spondylitis (6 45 48) Heart disease is thus very common and myocardial failure may occur independent of hypertension or valvular disease

Laboratory studies gave only some positive reactions of streptococcal or autoimmune etiology Some patients in this series had an elevated AST titre during the disease one of them repeatedly In three patients positive LE cell phenomenon was seen and all had a negative DNA antibody test reaction The literature contains some cases with a positive LE cell phenomenon (20 24 32 39) and some with antinuclear antibodies (20) The Waaler Rose test was positive in 5 patients and latex fixation in 4 in this series Electrophoresis and immunoelectrophoresis showed increased gamma globulins and immunoglobulins in 50 per cent of the cases. The rise in the gamma globulin level seems to be a constant feature in the acute phase of Takayasu s arteritis. The other immunologic studies, i e thyroid autoantibodies and Coombs reaction, were negative, except for one case of slightly positive Coombs tests Heart autoantibodies in the serum were not detected (23) Hirsch *et al* (20) studied arterial antibodies by the immunofluorescence method in five patients with negative results, but gamma globulin was found at autopsy in the aorta in one case In some patients of my series the excretion of hydroxyproline and the level in the serum were somewhat above the normal values but no conclusions could be drawn on this base

In this work the histological material was scanty and no specific diagnostic help could be got from muscle skin and temporal artery biopsies The good therapeutic response to corticoid common to all autoimmune diseases was seen in this series

As a summary of the above discussion of the possible autoimmune etiology of obstructive arteritis of Takayasu s type it may be said that the clinical picture sex distribution and dissimilar clinical picture of the sexes reaction to pregnancy, widespread arterial changes and »rheumatic infections in the history, cases of rheumatoid arthritis in the family and, most important, the concomitant autoimmune diseases of the patients are findings that make an autoimmune etiology probable The laboratory *tests and microscopic* studies were in most cases *»negative«, but three* positive LE cell phenomena positive Waaler Rose test and latex fixation in some patients, and elevated gamma-

globulins and immunoglobulins, are signs of a systemic process The lack of renal diseases in the series (with no renal biopsies) makes a disease like a serum sickness with glomerulonephritis improbable (30) An autoimmune disease would be a good explanation of this arteritis, although the evidences speaking for it are not confirming

Other etiological possibilities

Increased clotting tendency

One of the possibilities of explanations for the multiple stenoses and obstructions of the arteries would be an increased »clotting tendency» In patients of this series there were some abnormalities in the factors of clotting The *thrombocyte level* was high in 11 patients of 33, being from 350 000 to 704 000 per cu mm, and these patients had multiple stenoses Aggeler *et al* (1) have published the case of a patient with in occlusive process of the large vessels arising from the aortic arch of the aorta who had a marked thrombocytosis They thought that changes in the blood plasma or an increase in the number of circulating platelets could have been entirely responsible for this condition A *high fibrinogen level* has been investigated also earlier in Takayasu s arteritis (20, 24, 42) Over 60 per cent of our patients had a fibrinogen level of over 400 mg per cent High levels are commonly seen in patients with a high ESR, and the *etiology* or cause of this phenomenon is not known *»Clotting factor analysis» revealed normal factor concentrations in all* but two patients one had a somewhat lengthened euglobulin lysis time and one had a prolonged *recalcification time* caused by an unknown *anticoagulant in the* serum

It is possible that thrombocytosis and high fibrinogen levels with an increased clotting tendency and high blood viscosity were factors in the occlusive process Histologically there was intra-arterial thrombosis of fibrin-like material An unknown anticoagulant that was detected in one patient is known to occur in collagen diseases, and it may be a »compensatory» factor to thrombocytosis and a high fibrinogen concentration. Studies of the functions of thrombocytes should give more light on this problem.

Thyroid disease

The thyroid function was normal in all patients also in those with a cholesterol level of 350—399 mg per cent One patient had had a toxic nodular goitre many years before the arterial disease and it had been operated One patient had a slightly positive antithyreoglobulin antigen test but the rise in the titre was insignificant

Diabetes mellitus

Abnormal blood glucose tolerance was seen in 11 patients of the 24 that

were tested, two of them were under 30 years. This high incidence was surprising. Its explanation may be a reduced or slow insulin release because of arterial changes in the abdominal region, e g in the pancreas. The etiological or additive role of diabetes is to be taken into account, as is also the cause in patients with coronary diseases although the changes differ from the diabetic arteriopathy. Diabetic neuropathy, retinopathy or nephropathy were not seen in this series and none had a manifest diabetes mellitus.

Hypertension

Hypertension is a factor that accelerates changes in the arterial wall. Renal artery stenosis has been suggested as the most probable etiology of hypertension in Takayasu's arteritis (4 12 13 47). In this series there were 9 patients with uni- or bilateral renal artery stenosis (24 were examined) and six of them were hypertensive. One of the 15 patients without renal artery stenosis was transiently hypertensive. As suggested also by other workers it seems that hypertension is a secondary phenomenon based on arteritic changes with stenosis in the renal arteries. The possibility of microembolism from platelet fibrin thrombi also seen in connection with carotid thrombosis is not excluded as one ischaemic factor in the kidney leading to renal hypertension.

Tuberculosis

In this series there was one patient with old tuberculous spondylitis and epididymitis, and one with active tuberculous lymphadenitis. Mantoux tests in these and other patients were not convincing for tuberculous infection. It seems that tuberculous infection as an etiology of arteritis of this type is indirectly excluded. However, there is a possibility that a serious disease weakens the immunity reactions as has been seen in cases of cancer patients (21).

As a summary of other possible etiological factors of obstructive arteritis is that the thrombocytosis and elevated fibrinogen level of the plasma may be factors increasing the clotting but they are thought to be secondary to the systemic basic disease. Hypothyroidism was not seen in any patient. Hypertension was thought to be a phenomenon secondary to the arterial changes in the renal arteries. The frequency of pathologic blood glucose tolerance curves was surprisingly high. The etiological role of diabetes could not be excluded although a lowered glucose tolerance may be secondary to arterial changes. Tuberculosis was improbable because only one patient had an active tuberculosis and the Mantoux tests were at a normal level. There was no positive syphilis serology.

Comments on treatment

The therapy of patients with obstructive arteritis of Takayasu's type

has been drug therapy with corticoid and anticoagulant and/or surgical therapy The patients with a high ESR and systemic symptoms were given corticoid therapy and almost all were treated with anticoagulants Case 1 was treated with corticoid for one year Therafter the disease has been inactive during 6 months In one case the systolic murmurs in the neck were much weaker after some weeks of cortisone therapy with general improvement In one case the size of the heart has increased and myocardial insufficiency has been resistant to treatment It was seen, as has been observed also elsewhere, that corticoids and anticoagulants in the acute phase and anticoagulants alone in the chronic phase are of value in the treatment The duration of the disease ranges from 6 months to 14 years (16) or even more (10) In this series the longest duration was 12 years The disease seems to change from an active to an inactive form in most patients spontaneously or after treatment with corticoid. Anticoagulant therapy seems to be indicated in all cases in the active phase to prevent thrombotic complications, its value in the chronic phase may be smaller In selected cases with severe ischaemic symptoms and in cases with syndrome of subclavian steal surgical correction is indicated.

SUMMARY

Clinical roentgenological and laboratory studies on 36 patients with obstructive arteritis of Takayasu's type were reported. By sex the patients were 23 women and 13 men, aged from 19 to 53 years (except for one patient of 63 years). Under 40 years were 18 patients, 15 of them women.

Family history, smoking habits, effect of child bearing and previous and concomitant diseases were reported. Four patients had ankylosing spondylitis, one had SLE and one myasthenic syndrome. The most common symptoms were general malaise and fever, claudication of the arms and legs, vertigo, dizziness and visual disturbances. Anginoid pains of the chest, dyspnoea and hypertension were common. Pains and swelling of the joints and bursae were common.

Changes in the arteries were multiple in all cases. The most common sites were the carotid and subclavian arteries, iliac arteries, aortic arch and abdominal aorta. In nine cases there was a stenosis of the renal artery, in four of them bilateral. Six of these patients had hypertension. In one case the superior mesenteric artery and in one case the pulmonary artery were affected. Calcifications were present in seven cases. The syndrome of subclavian steal was seen in four cases and in one of them it possibly was bilateral.

Auscultatory, electrocardiographic and/or roentgenographic signs of heart disease were seen in 24 patients, including 3 cases of myocardial infarction, one of pericarditis and six of slight aortic insufficiency. The others had a systolic murmur in the aortic area and/or changes in the electrocardiogram. One patient suffered from progressive myocardial insufficiency.

In the laboratory studies the distribution of blood groups showed some predominance of the A Group. ESR was 41 mm or more per first hour in 52 per cent of cases. Hypochromic anaemia was diagnosed in 33 per cent of the patients. The leucocyte count was normal with exception of 8 cases with a slight elevation, and the percentile eosinophilia was from 5.5 to 9 per cent in five cases of the 33 studied. Serum cholesterol, PBI, uric acid, SGOT, SLDH and alkaline phosphatase, potassium, sodium, chloride, calcium and phosphorus were at the normal levels. An elevated gamma-

globulin value was seen in the protein electrophoresis of 14 patients (relative proportion over 20 per cent) The fibrinogen level of serum was 400 mg per cent or more in 22 cases, or in 63 per cent of the patients

Syphilis serology was negative The AST value was elevated transiently in nine cases, ASta was normal The Waaler-Rose test was from 32 to 180 in five, latex fixation was positive in four, LE cell phenomenon was positive in three cases but DNA antibodies were not detected In one case a slightly positive antithyroglobulin antibody titer and in one case a slightly positive Coombs reaction were seen Immunoelectrophoresis revealed elevated immunoglobulin levels in 18 cases The hydroxyproline level of serum or its excretion into the urine were increased in four patients, without correlation with the activity of the disease Heart antibody test was negative

None of the patients had a manifest diabetes mellitus, but a pathologic glucose tolerance test was seen in 11 patients of the 24 studied Vitamin A absorption was normal No increased tuberculosis sensitivity was seen The biopsies (temporal artery, muscle, skin) revealed in some cases changes associable with »collagen» disease, but were not more definitely diagnostic

In the discussion, facts speaking for autoimmune etiology of the presented disease were rheumatic disease in the family in some cases, recurrent streptococcal infections in most of the patients, the concomitant occurrence of autoimmune disease, the clinical picture in the acute phase, the periodicity, the effect of gravidity, the multiplicity of arterial changes, the positive laboratory findings and biopsies in some cases, and a good response to corticoid treatment Other etiological possibilities were also discussed Thrombocytosis and a high fibrinogen level were thought to be secondary to the basic disturbance The pathological glucose tolerance was also thought to be a phenomenon secondary to the arterial disease Thyroid diseases and tuberculosis were not etiological diseases Hypertension, present in six cases, was only slight or moderate A short comment on treatment was made

It is concluded that obstructive arteritis of Takayasu's type is not a rarity Most of the patients are women The diagnosis is to be made by clinical criteria, angiography and laboratory studies in acute and chronic phases None of the studies is specific It is possible that this clinical picture is caused by diseases based on different basic disturbances, and a disorder of the autoimmune mechanism seems to be one possibility

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APPENDIX

Case reports

Case 1 — Female, b 1945 — The patients mother had bronchial asthma The patient had tonsillitis 3 times at the age of 7 and 12, but no joint symptoms Occasionally there has been pain in the lumbal spine Since May 1964 she had suffered from pains in the left shoulder, numbness and coldness of the arm, general malaise, irritability and thirst, high ESR, and pulselessness of the radial artery of the left hand Murmurs were audible over the left carotid and subclavian arteries and the abdominal aorta Under treatment with corticoid and anticoagulant the murmur became fainter, but otherwise the findings were unchanged in Dec 1965 when this treatment was stopped because of gravidity She delivered in Aug 1966 without complications and with no exacerbation of the disease

Case 2 — Female, b 1941 — Vision had been poor in the left eye for over ten years 1960 a systolic bruit was heard in the neck in a health examination, ESR was 45—50 per first hour, and there was a systolic murmur over the aortic area, a thrill over the left carotid artery and a weak left radial pulse The papilla in the left eyeground was atrophic, and peripherally arteriovenous anastomoses and aneurysms were seen. She was put on anticoagulant and corticoid therapy for some weeks — In 1964 she had no subjective symptoms The left axillary and radial arteries were weak and there was a rough systolic bruit over the right radial and subclavian arteries Aortographic studies were refused No treatment was instituted

Case 3 — Female, b 1941 — In 1962 the patient had intensive pains under the jaw and in the neck 3—5 times daily especially after drinking cold beverages. She was tired and had a high ESR. The symptoms subsided for some months but worsened again in 1965 and ESR was 112 mm per first hour Bruits were heard in the aortic area of the heart, over the right subclavian artery over both carotid bifurcations and in the epigastrium X-ray changes typical to ankylosing spondylitis were seen. Aortography revealed changes in the right brachiocephalic trunk. Response to corticoid and anticoagulant treatment was good

Case 4 — Female, b 1939 — This patient had had recurrent tonsillitis and pains in joints of arms and legs from the age of 7 to 12 In 1963 she complained of aching pains in the hip joint and lumbal area and of a transient neck stiffness Findings at this time were a high ESR, anaemia, general malaise pulselessness of the left radial artery and a persistent sterile pyuria. There were pulsations in the neck, murmurs over the carotid and subclavian arteries, abdominal aorta and femoral arteries and a thrill over the bifurcations of the carotid arteries X-rays revealed changes typical to ankylosing spondylitis. — Corticoid and anticoagulant treatment were given. In 1966 after 2 years treatment, about the same findings were made the left carotid artery was very weak and the radial artery pulseless. After two weeks without corticoids the ESR rose to 58 mm per first hour and the general malaise resurred She is on continued corticoid and anticoagulant treatment.

Case 5 — Female b 1937 — The brother of the patient has a severe juvenile rheumatoid arthritis She has had recurrent tonsillitis (tonsillectomy in 1954) followed by general malaise, fever elevated ESR, anaemia intensive pains in the interscapular region of the thorax and palpable radial pulse These disease episodes recurred at some months better intervals In 1958 there was a parenchymal infiltration in the lung but no bacteriological evidence of tuberculosis The radial arteries were not palpable. In 1962, after delivery she had a rheumatic fever with fever of 39 C, high ESR, joint swellings and radial pulselessness The carotid arteries pulsated weakly and a thrill was felt over the left one A systolic bruit was heard from the aortic area of the heart to the neck, over the both carotid arteries abdominal aorta and femoral arteries Changes typical of ankylosing spondylitis were seen in s-i joints — Corticoid and anticoagulant were given with good response but after one year the patient stopped this treatment, with recurrence of high ESR and some general symptoms but she managed to do household work

Case 6 — Male b 1935 — There was no history of tonsillitis or joint symptoms In 1962 he experienced abruptly coldness of the legs and pulselessness of the posterior tibial and dorsal pedis arteries AST was 560 All arteries were firmer than normal ones A systolic bruit was heard over both carotid arteries and over the abdominal aorta The femoral arteries pulsated weakly A muscle biopsy (during lumbal sympathectomy) revealed degeneration and organizing thrombosis in the muscular arteries. — He was treated by lumbal sympathectomy and anticoagulant, with no change during 2 years.

Case 7 — Male b 1934 — When 7 years of age the patient had tuberculous spondylitis In 1957 there were pains in the right arm, in 1959 fits of anopsia, vertigo and headache in 1961 and 1962 rightsided transient facial paresis in 1962 weakened pulsations thrill and murmur over the left carotid artery and both subclavian arteries, and an elevated ESR Endarterectomy of stenosed carotid arteries was made Bilateral endarterectomy of the subclavian arteries with good result was performed in 1963 PAD was thrombosis In 1964 there were no symptoms from the brain, the carotid arteries were weak, the right radial artery pulseless, and the femoral arteries not palpable Systolic bruits were audible on the right in the neck and loin. Blood pressure could not be measured in the arms but in the leg it was 170 systolic and 105 diastolic Anticoagulant and antituberculous treatments were given.

Case 8 — Female b 1933 — She had had tonsillitis at the age of 13 In 1959 she had tonsillitis followed by rheumatic fever with purpura, high ESR and AST of 2500 maximally Thereafter there were joint swellings and bursitis (wrists and interphalangeal joints) recurrently and PAD from a biopsy of the joint synovia was rheumatoid arthritis (a rich round cell infiltration and some fibrinoid necroses) Pharyngitis and tonsillitis recurred once or twice yearly with AST elevations and joint and/or bursitis symptoms In 1962 there was a pericardial friction and pain in the left shoulder A thrill and a murmur was heard over the left subclavian artery a slight systolic murmur over the aortic area of the heart and the right side of the neck. Aortography showed stenosis and unevenness of the lumen of the left subclavian artery In 1964 the blood pressures of the arms were hypertensive 190/130 and 180/120 Renal arteriography was normal. Half a year later the pressures were 155—185/110—80 — This patient received antibiotics and antirheumatic drugs

Case 9 — Female b 1931 — Since childhood the patient had tonsillitis with slight joint symptoms every year Hydrops of the left knee was treated from 1954 to 1962. Since 1950 she complained of numbness and weakness of the left arm, with bluish and cold fingers when working. Blood pressure was then normal, 120 mm Hg systolic. In 1955 the left radial artery was weak, and

a systolic murmur was heard in the neck In 1956 the blood pressure in the right arm was 230/140, in the left arm 150/125, and in the legs 280/190 and 260/160 The eye grounds showed pigmentation in the periphery Stenosis of the left subclavian artery was seen in the aortography in 1962 In 1964 a thrill and murmur were detected over the right carotid and subclavian arteries, the left carotid artery was weak In the upper abdomen there was a systolic bruit Aortography revealed great changes This patient was given anticoagulant and antihypertensive treatment.

Case 10 — Female, b 1929 — When 17 she had tonsillitis since the age of 19 general malaise, vertigo and tiredness of the arms, and the last few years claudication of the legs, headache and tachycardia A high ESR, joint tenderness and pulselessness, were observed in 1958 In 1961 she had fever and joint swellings for many weeks and pain in the interscapular area Febrile periods were treated with corticoids with good response ESR was between 45 and 95 In 1964 the arteries were stiff the radial arteries did not pulsate and a systolic bruit was noted over the carotid arteries with a thrill at their bifurcations Aortography showed stenoses and obstructions of arteries — Treatment was corticoids and anticoagulant

Case 11 — Female, b 1926 — Since the age of 17 there was acute tonsillitis twice a year up to the age of 30 At 17 she had rheumatic fever After parturition in 1955 at age of 29 she had febrile episodes, high ESR and joint symptoms for about 2 months Cholecystectomy was performed in 1961 In 1963 she had intensive pains in the lumbal area general malaise fever, high ESR, anginoid pains pain in the neck when eating, and vertigo In 1964 there was an abnormal pulsation in the neck the right radial artery was weak the right axillary artery was dilated and there was a thrill and murmur over it Murmurs were heard in the neck over the abdominal aorta and both femoral arteries Narrowed stenotic arteries were seen in aortography — After treatment with corticoid and anticoagulant the ESR fell and subjectively the patient was better, but anginoid pains and myocardial insufficiency were unchanged, the heart is increased in size and the blood pressure elevated The prognosis seems to be very poor

Case 12 — *Male, b 1924* — *The patient is a heavy smoker* He had tonsillitis in 1943 and 1960 *After the last case he complained* of continual tiredness headache claudication of the left arm and impotency A year later the left radial pulse was very weak and the electrocardiogram revealed changes of myocardial infarction In 1961 there were claudication of the legs and visual disturbances A slight atrophy of the muscles of the left leg and calf were seen 1963 The left carotid artery was weak and a murmur was heard over both carotid arteries The left radial artery and femoral artery and the peripheral arteries of both legs were not palpable A slight bruit was audible over the abdominal aorta X-rays showed calcification of the abdominal aorta and iliac arteries with obstruction of the left iliacal artery — The patient was treated with anticoagulant The finding has remained unchanged

Case 13 — Female b 1921 — Before the age of 24 the patient had tonsillitis every autumn At the age of 28 she was treated for a period of agrypnia In 1954 she had intensive pains in the left olecranon for about two months and in 1955 branching pains in the chest for some days These pains lasted and worsened during work Two years later the same symptoms and additionally pulselessness of the left radial artery were detected In aortography the left subclavian artery was replaced by a teflon graft, but this was open for only 3 months Thereafter there has been tiredness and coldness of the left arm leftsided headache which increases during working with the left arm and impaired vision The left foot has become cold and parestetic — She has been treated with anticoagulant since 1960 — In 1964 her arteries were stiff and the bifurcations of the carotid arteries were nodular

and hard The right carotid artery was weak, the left axillary and radial arteries were not palpable the femoral arteries were weak. A short murmur was audible over abdominal aorta. Both dorsal pedis arteries were palpable In the left eyeground the changes of an old chorioretinitis were seen.

Case 14 — Male b 1923 — He had had tonsillitis several times and tonsillectomy was performed in 1960 After this he had symptoms of arthritis and received treatment (gold) for three months, with recovery Later there again were swelling and pain in the hands and stiffness in the lumbal region and neck The pulses of the radial arteries were normal. In 1963 both arm became powerless and the radial pulses were weak In 1964 all the symptoms worsened, he lost weight, the muscles waisted and ESR was elevated The diagnosis of ankylosing spondylitis was made In 1965 the subclavian, axillary and radial arteries were not palpable The carotid arteries were firm, with a systolic bruit, and a murmur was heard in the epigastrium and over both femoral arteries Arteriography revealed great changes in the arteries At operation it was seen that the right subclavian artery was inflamed and the surrounding periarterial tissue was fibrotic From the lumen a white mass was removed which at microscopic examination showed organizing and organized thrombus covered with thickened arterial intima. Some inflammatory cells were seen. After operation the right radial artery has been palpable — After operation the patient was treated with anticoagulant and anti inflammatory drugs.

Case 15 — Male b 1921 — For many years the ESR had been about 25 mm per first hour In 1964 joint swellings and aching occurred and he was given gold therapy Some months later in the same year there was claudication of the left leg. In 1965 a muscle biopsy showed angiitic changes in the small vessels, with lymphocytic infiltrations the picture was that of SLE. A murmur was heard over the right carotid and subclavian arteries and a systolic bruit in the upper epigastrium and the femoral arteries, the peripheral arteries of the legs were not palpable Aortography showed a slightly later filling of the left subclavian and carotid arteries and changes in the femoral artery and its branches — The patient was treated with chlorochin and vasodilatatory drugs.

Case 16 — Female b 1921 — In 1951 at the age of 30 the patient had aching pains and swellings of wrists and knees for about 3 weeks In 1959 there was an attack of chest pain. In connection with operation for cholelithiasis in the following year the ECG showed changes due to an old myocardial infarction ESR was elevated continuously An aneurysmal dilatation of the descending aorta was seen in radiograms. — In 1964 there was an abnormal pulsation in the right side of the neck, the left carotid artery was weak and there was a thrill and systolic murmur A murmur was heard over both subclavian arteries abdominal aorta and femoral arteries The peripheral arteries of the legs were palpable In chest x-ray an aneurysmatic dilatation of the descending aorta was seen. ECG revealed an old inferior myocardial infarction, and aortography showed marked changes in the aorta and its branches — The patient was treated with anticoagulant and vasodilatatory drugs In check-ups in 1964 1965 and 1966 the situation was about the same

Case 17 — Female b 1918 — At the age of 15 tonsillectomy was performed after many years of recurrent tonsillitis In 1938 at the age of 20 she had joint swellings and pains during 6 weeks A toxic nodular goitre was removed in 1950 and amputation of the uterus was made in 1964 During the last few years she had complained of tiredness, impaired memory vertigo headache and hoarsening of the voice — At examination in 1964 the right carotid artery was weaker than the left and a murmur was heard over both carotid arteries Over the right subclavian artery there was a thrill The right radial and axillary arteries the right femoral artery and the peripheral arteries of legs

were not palpable Over the abdominal aorta there was a bruit. Aortography showed obstructions, and endarterectomy of the brachiocephalic trunk and left subclavian artery was attempted The vessels were stiff and scarred, and obstructed by a throubus — The patient has been treated with anticoagulants continuously Her abdominal pains have worsened

Case 18 — Female, b 1918 — From the age of 37 to 43 she had tonsillitis every year and for 15 years Raynaud s phenomenon of the hands and feet. During the past 4 years she had pain in the shoulders and tiredness of the arms during work In 1964, there were anginoid pains radiating from the chest to the neck, mouth and shoulders, especially in the morning and lasting from minutes to hours She lost weight The blood pressure had been about 200 mm Hg systolic for some years — The arteries were stiff and hard and an abnormal pulsation was seen in the right supraclavicular region The left carotid artery was weak, the femoral arteries also but peripheral arteries of the legs were palpable A harsh murmur was heard over both carotid arteries especially the right one, and over femoral arteries In the upper epigastrium and over femoral arteries there also was a murmur — The patient was treated with anticoagulant and her symptoms have been fluctuating

Case 19 — Female, b 1918 — At the age of 15 she had poliomyelitis from which she recovered For many years there was stiffness and light swelling of joints of hands and foots in the morning Claudication of the left calf first occurred in 1958 at the age of 40 years and during the last 3 years there had been pains in both calfs legs and abdomen after 200 meters walk. She felt anginoid pains in the chest In 1964 an obstruction of abdominal aorta was diagnosed by angiography — The arteries were hard, and the left radial artery pulsated weakly There was a murmur in the upper abdomen, but the aorta was not palpable — She was treated with anticoagulants, but without effect. Her abdominal pains were thought to be symptoms from abdominal angina

Case 20 — Male, b 1918 — In 1955 he had rheumatic fever, at the age of 37, with recovery In the same year he had severe headache and possible encephalitis Headache, nausea and vertigo recurred 1956 and now the left radial artery was not palpable and a systolic bruit was heard over the right carotid artery He received corticoids for some weeks with good result. Again in 1962 there was a period of headache vertigo and claudication of the right hand Anticoagulant treatment was begun In 1963 he suffered leftsided hemiparesis of short duration, after which there was no headache, but he had anginoid pains and the claudication of the arm got worse — In 1964 the left arm was weaker than the right. The arteries were hard, and only the pulsations of the right carotid, right subclavian and right radial arteries and of the femoral arteries were felt. A systolic murmur was heard in the neck on the left side over the abdominal aorta and the left femoral artery Aortography showed marked changes. — He was treated with anticoagulant but with no change in the symptoms during follow-up time of one year

Case 21 — Male b 1917 — There had been recurrent tonsillitis from child to the age of 20 without complications Three times during the past 10 years he had pain and swelling of the left knee lasting 2—3 days — About 10 years ago there were pains in the back and in the interscapular region, anginal pains in the chest, general malaise and a high ESR during 3 months. No diagnosis was made and he received no treatment. For about 2 years he had anginal pains in the morning and general malaise Both arms were tired he had headache and once a collapse In 1965 the arteries were found to be hard Both carotid arteries pulsated only weakly and a thrill and a murmur were audible over them The axillary and radial arteries were hardly palpable the abdominal aorta pulsated normally the femoral arteries were hard A systolic bruit was heard on the left side of the epigastr-

ium and over the femoral arteries Arteriography exhibited arterial changes with the subclavian steal syndrome possibly bilateral. — This patient is on anticoagulant treatment and endarterectomy of the subclavian arteries will be attempted later

Case 22 — Female b 1917 — At the age of 25 patient's right hand became cold and blue and two fingers were amputated. Some weeks thereafter the symptoms appeared in the left hand. Sympathectomy was of help One year later the symptoms became worse. There was a slight anisochoria tiredness, claudication of arms and constant vertigo Recurrent pyelonephritis was treated with sulfonamides In 1964 she was tired and thin The movements of the right hand were stiff and the skin and muscles were atrophic in both arms and hands. The left calf was 2 cm thinner than the other The arteries were hard Both carotid arteries pulsated well but a harsh systolic murmur was heard The left radial and axillary arteries were pulseless. The abdominal aorta pulsated weakly and there was a systolic bruit over it. In arteriography the syndrome of subclavian steal was seen — She was given anticoagulant treatment

Case 23 — Male b 1916 — The patient's father mother and two brothers have rheumatoid arthritis In 1942 when 26 he got slight joint swellings bursitis and pains. These symptoms worsened nine years later but the diagnosis of rheumatoid arthritis was uncertain After a better interval the joint symptoms recurred in 1958 and a duodenal ulcer was diagnosed In 1962 the right arm became cold and white and the radial pulse was not felt Rightsided endarterectomy of the subclavian artery was made and anticoagulant treatment continued. In 1964 there were some joint symptoms and tiredness of the right arm. An acute phase occurred in 1966 with swelling of the bursae of the wrists and ankles and a high ESR. Corticoid treatment was of good effect. No x-ray changes of the joints were seen. — This patient was given continuous anticoagulant treatment and received corticoids during the acute phase

Case 24 — Male b 1916 — In 1964 there occurred a transient hemiplegia of some minutes duration. Thereafter he suffered from dizziness and claudication of the legs In 1965 he had a rightsided hemiplegia but made rather good recovery The left carotid artery was weak and a systolic murmur was audible over it. The radial artery on the left side was not palpable the peripheral arteries of legs were felt. The arteries were hard The eyegrounds were normal. — He was treated with anticoagulants

Case 25 — Male b 1914 — When 20 years old he had a severe case of scarlatina. In 1963 there occurred claudication of legs and anginoid pains in the chest. In 1964 he lost the vision of the right eye for periods of one-half to 3 minutes The ESR was high, an infiltration was seen in one of the lungs no bacilli detected, antituberculous treatment was given without effect. He was subfebrile, a murmur was present over the carotid arteries and the femoral arteries. X-rays of the lung showed some old fibrosis and enlarged mediastinal lymphnodes. Biopsy revealed tuberculous lymphadenitis — He was treated with anticoagulant and antituberculous drugs with good effect.

Case 26 — Female b 1914 — She had tonsillitis and pneumonia in 1945 Since 1950 there were anginoid pains in the chest and a buzz in the head on the right side A high ESR and pyuria occurred in 1965 the left radial artery was not palpable During winter of 1964—1965 she had severe vertigo The muscles of the left arm were atrophic The arteries were hard and a thrill was palpable over both carotid arteries and the right subclavian artery The left radial artery was hardly palpable The femoral arteries and the peripheral arteries of the legs were felt, but a murmur was heard over the abdominal aorta and femoral arteries. Aortography confirmed great changes and the syndrome of subclavian steal. — Waaler-Rose and latex fixation tests were positive — She received anticoagulant and antirheumatic drugs, with no clear effect.

Case 27 — Male, b 1911 — In 1943 he had a fever of many weeks duration without a diagnosis, but malaria was suspected In 1959 there occurred anginoid pains and pulselessness of the radial artery He had fever, leucocytosis and a high ESR In aortography a stenosis was seen in the left subclavian artery, the diagnosis of »pulseless disease was made, and he was on corticoid and anticoagulant therapy same weeks, with good recovery — In 1962 the anginoid pains recurred and there was vertigo and claudication of the legs After a tonsillitis in 1964 he continually had fever and anginoid pains in the chest — The arteries were hard, the pulsations of the right carotid, axillary and radial arteries were weaker than on the left, and there was a murmur over the right carotid artery He was treated with antibiotics and anticoagulant, the fever subsided and the pains disappeared

Case 28 — Female b 1908 — When 20 years old she had a period of fever, joint swellings and high ESR. In 1953 at the age of 45, she had joint swellings and fever In 1963 there was eczema and claudication of the legs and arms In 1965 a diagnosis of SLE was made with positive LE cells, and with pleuritis, pericarditis and nephropathy The arteries were stiff and the carotid bifurcations hard The left radial artery was weak the abdominal aorta was hardly felt, and the femoral arteries were weak A murmur was heard over the carotid arteries abdominal aorta and femoral arteries — This patient was treated with corticoid but the prognosis is poor

Case 29 — Male b 1920 — The patient had scarlatina at the age of 11 For 20 years he had symptoms of duodenal ulcer In 1950 at the age of 30 he got anginoid, pains vertigo and headache and in 1961 a leftsided hemiplegia with recovery Thrombosis of the right carotid artery was seen in angiography In 1963 his right carotid artery was not felt and the right radial artery was *barely palpable* Murmurs were heard in the neck over the subclavian arteries In 1954 endarterectomy of the brachiocephalic trunk was made The periarterial tissue was scarred and fibrotic The vessel was filled with a thrombus The result of the operation was good his headache disappeared and the right radial artery was stronger Anticoagulant treatment *was continued*

Case 30 — Female b 1929 — At the age of 24 she had tonsillitis with high fever Since the age 15 her left arm has been cold and white, and at the age of 22 the radial pulse was not felt. In 1963 at the age of 34, also the right radial artery became pulseless, and the coldness and tiredness *of both arms worsened* After her third delivery in 1963 she suffered from heavy pains in the hands and the interscapular region and an eye inflammation. The ESR was high She reveived corticoid therapy with good response In 1965 the symptoms recurred. Both carotid arteries pulsated only weakly and the radial pulses were not felt. There were no pulsations in the temporal arteries A thrill and a harsh systolic murmur were heard over the carotid and subclavian arteries and over the abdominal aorta and femoral arteries too Corticoid and anticoagulant therapy was instituted One year later the symptoms were about the same, but the inflammatory reactions had subsided — Corticoid and anticoagulant therapy was continued

Case 31 — *Female, b 1921* — *Her mother* had rheumatoid arthritis In April 1965 she felt tenderness in the neck and in May 1965 a severe ache The ESR was high, and she had fever and general malaise In June there was vomiting and dizziness These symptoms disappeared during corticoid therapy A new *exacerbation occurred in September* with general malaise, fever, pains in the neck and the heart, and tenderness in the left shoulder Both carotid arteries were tender and weakly pulsating and there was a systolic bruit. Aortography showed changes in the aortic arch — With corticoid and anticoagulant therapy the pains and tenderness disappeared

Case 32 — Male b 1913 — This patient was treated in hospital in 1962 for a drug fever

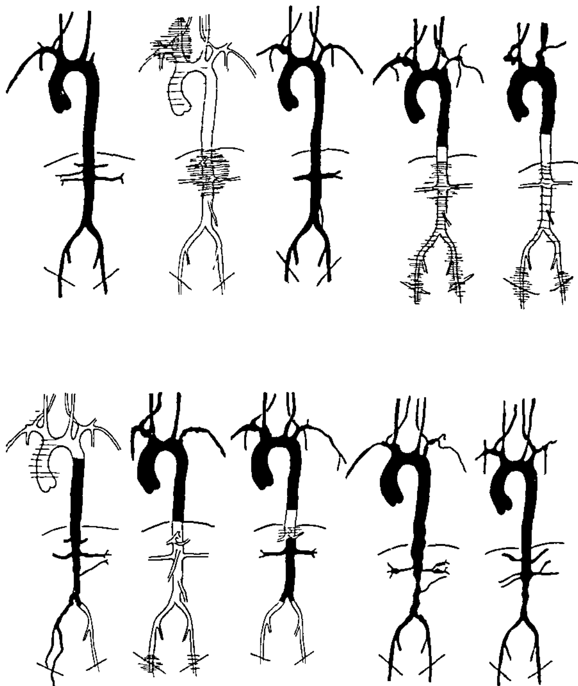
and pyelonephritis. In 1963 at the age of 44 he had tachycardia and slight anginal pains blood pressure was elevated. In 1964 a rightsided hemiplegia occurred during sleep with good recovery Blood pressure was then 210/120 Thereafter he had fever a high ESR, CRP 3 + and AST 320 In 1965 he had claudication of the legs and electrocardiographic changes indicating posterior myocardial infarction. The arteries were hard the left radial, popliteal and dorsal pedis arteries were not palpable a systolic murmur was heard over the carotid arteries in the upper epigastrium and over both femoral arteries — He received anticoagulant therapy and coronary vasodilators

Case 33 — Female b 1914 — She had had tonsillitis once At the age of 44 in 1958 there was general malaise slight dizziness and diplopia. Takayasu's arteritis was diagnosed In 1965 she again was tired and had headache tachycardia, coldness of arms and elevated ESR In 1966 there was some atrophy of skin and muscles The left radial artery was not felt. A systolic bruit was heard over the carotid subclavian and femoral arteries and in the upper epigastrium. Waaler-Rose test was 180 — She gets anticoagulant therapy and corticoid with good response

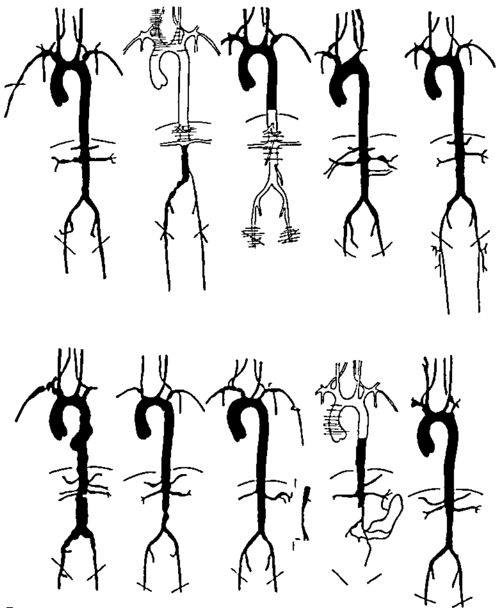
Case 34 — Female b 1948 — When 10 years of age the patient had bronchial asthma, and afterwards slight symptoms of it. In 1964 at the age of 16 she noted tiredness of the legs after work and difficulties in walk-up stairs One year later she had to stop working because of weakness of the arms and legs. She felt no muscular pain or tenderness but could not get up from bed. Myasthenia gravis was suspected, and the Tensilon® test was positive Her face was somewhat oedematous no palpebral ptosis was noted No muscular atrophy was present. Electromyography revealed a myasthenialike syndrome no effect of Tensilon. The electroencephalogram was normal. — There was a thrill over the left subclavian artery and a harsh systolic bruit over the left carotid subclavian and femoral arteries and in the upper epigastrium. The changes were confirmed by aortography The diagnosis of the Neurological Clinic was myasthenic syndrome — She is on anticoagulant treatment and physical therapy

Case 35 — Female, b 1937 — At the age 15 to 17 years the patient suffered from pain and swelling of the fingerjoints When 19 she had many febrile periods of some weeks, without a diagnosis During pregnancy in 1960 she had an elevated blood pressure and a slight proteinuria, which continued after delivery In 1964 there were breathlessness transient diplopia and pains in the back and epigastrium. Raynaud's phenomenon of the hands was seen. The skin was pigmented brown The right carotid artery and radial artery pulsated weakly and there was a thrill over the right subclavian artery Compression of the right carotid artery caused diplopia. Renal biopsy revealed thickening of the basal membranes in some glomeruli, arteriolar thickening hyalinization and endothelial proliferation. — She received antihypertensive drugs and corticoid with improvement.

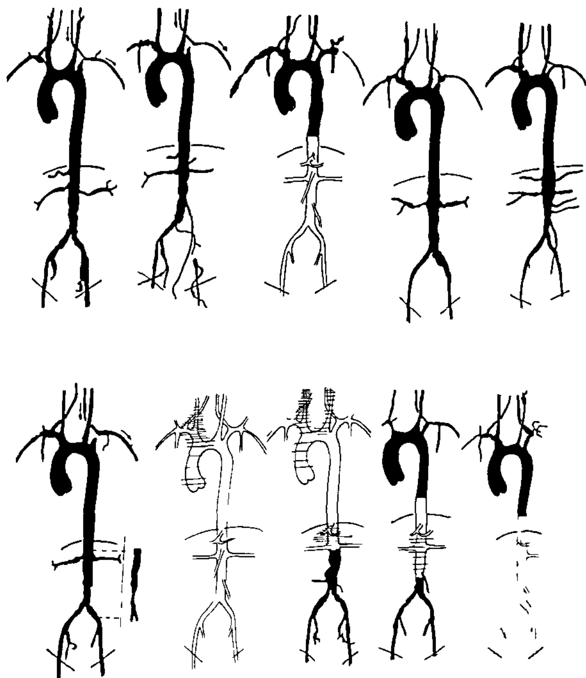
Case 36 — Female b 1931 — This patient had tonsillitis once In 1963 at the age of 29 she felt pains, numbness and aching in the left arm. One year later there was aching in the neck and chin, and the tongue was sometimes painful. ESR was high and there were leucocytes in the urine The symptoms subsided during antibiotic therapy but in 1965 there again occurred back pain, fever pain and aching in the left shoulder and arm, in the neck and in the chest. The ESR was high. The arteries were thick and the left brachial and radial arteries were weak and pulsated later than the right ones. A systolic bruit was heard over both carotid arteries, subclavian and femoral arteries and in the upper epigastrium. In angiography the subclavian steal syndrome was diagnosed on the left. — During anticoagulant and corticoid therapy the symptoms subsided.



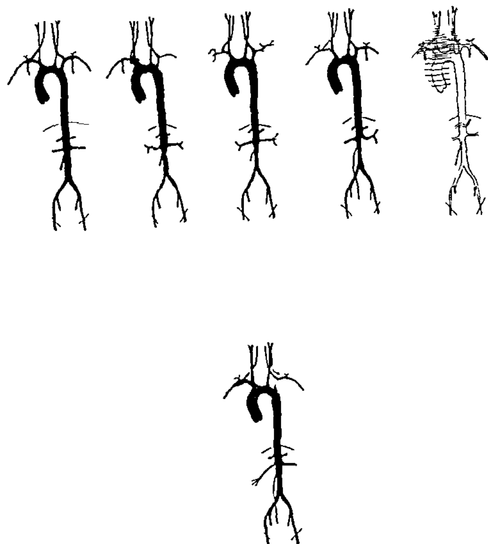
Figures 1—10 — Arteriographic findings in the patients with obstructive arteritis of Takayasu's type Auscultatory findings are presented by horizontal lines in the areas not visualized in the arteriography



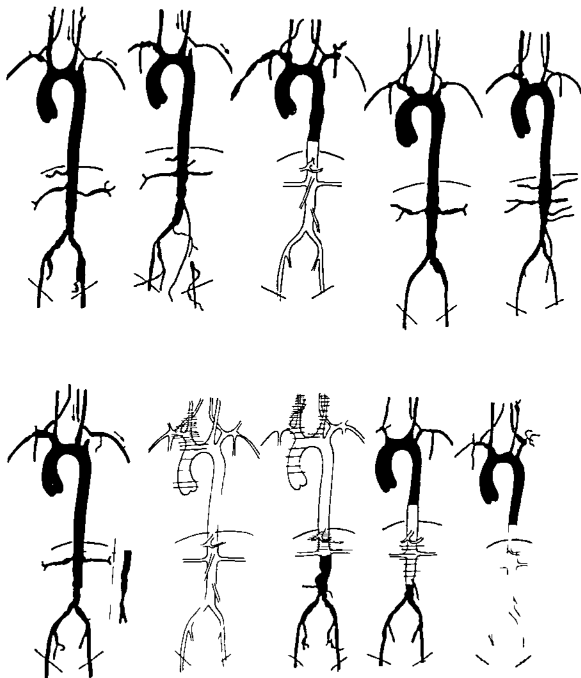
Figures 11—20 — Arteriographic findings in the patients with obstructive arteritis of Takayasu's type. Auscultatory findings are presented by horizontal lines in the areas not visualized in the arteriography.



Figures 21—30 — Arteriographic findings in the patients with obstructive arteritis of Takayasu's type Auscultatory findings are presented by horizontal lines in the areas not visualized in the arteriography



Figures 31—36 — Arteriographic findings in the patients with obstructive arteritis of Takayasu's type. Auscultatory findings are presented by horizontal lines in the areas not visualized in the arteriography



Figures 21—30 — Arteriographic findings in the patients with obstructive arteritis of Takayasu's type. Auscultatory findings are presented by horizontal lines in the areas not visualized in the arteriography

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[illegible]	Time of the study	ESR mm per first hour	Haemoglobin g per 100 ml	Leucocytes per cu mm	Eosinophils per cent of leucocytes	Thrombocytes 1000 per cu.mm	Serum Fe γ g per 100 ml	TIBC γ g per 100 ml	Serum cholesterol mg per 100 ml	PBI μg per 100 ml	Total protein g per 100 ml	albumen per cent	α_1-globulin, per cent	α globulin per cent	β globulin per cent	γ-globulin per cent	Fibrinogen mg per 100 ml	AST	ASta	Latex fixation test	Waaler Rose test	LE cells	Blood Group	Hydroxyproline in serum, μg per ml	Hydroxyproline excretion into urine mg per day
9	1964 196. 1965	51 11 11	12 8 11 9	6900 6600	1 0 1 0	238 32.	76	346	208 240	5 1	8 0 8 0	51 4 56 0	5 8 4 0	10 2 9 0	11 8 13 5	20 8 17 5	370	320 250	1 0 1 0	—	— —	—	B Rh+	0 91	20 3
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964	22	11 8	7500	4 0	389									11 8	17 4	44	2 0	<1 0	—	—	—	A Rh—	1 52	24.3
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SUPPLEMENTUM 469

THE KRISTIANSTAD SURVEY II

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with special reference to comparison with an adequate control group

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KRISTIANSTAD 1967

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Supplementum 469

From the Department of Medicine (Head Henning Lindholm, M D)
the Department of Ophthalmology (Head Herman Grönvall, M D)
and the *Department of Roentgenology (Head Nils Frostberg, M D)*
Kristianstad General Hospital, Kristianstad Sweden

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Supplementum 469

From the Department of Medicine (Head Henning Lindholm, M D)
the Department of Ophthalmology (Head Herman Grönvall M D)
and the Department of Roentgenology (Head Nils Frostberg, M D)
Kristianstad General Hospital, Kristianstad, Sweden

THE KRISTIANSTAD SURVEY II

Studies in a representative adult diabetic population
with special reference to comparison with an adequate control group

BY

SVEN E. NILSSON, JAN E. NILSSON,
NILS FROSTBERG AND TRYGGVE EMILSSON

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Translated by I James Brown

Contents

I Introduction and purpose of the investigation

This investigation is concerned with an analysis of clinical, anthropometric and *laboratory findings in a diabetic* population consisting of practically all diabetics aged 20 to 79 years and residing in a defined geographical area. Values obtained in a corresponding *investigation of a representative* sample of the general population by the same methods (NILSSON et al 1964) served as controls. In the analysis due consideration was given to the patients' ages and the duration of the disease.

The main aspects elucidated are as follows:

1. *Variation of body weight of diabetics* with duration of the disease and then particularly of the ratio between body fat and fatfree weight.
2. The specific association of certain complications with diabetes judged mainly by comparison between diabetics and the above mentioned controls and by correlation with *the duration of diabetes*.
3. Comparison between diabetics with *and without diabetic retinopathy* regarding anthropometric data, therapy, regulation, presence or absence of other complications and genetic factors.
4. Various clinical and laboratory data elucidating vascular, neurological and ophthalmological status of diabetics who have had the disease for different periods, who have received different types of treatment and with varying degree of regulation.

The investigations were carried out at the departments of internal medicine, *roentgenology and ophthalmology*, Central hospital, Kristianstad, in cooperation with colleagues there, to whom we express our sincere gratitude for smooth cooperation.

The data were treated in an electronic computer by Lector TORGIL LEKMAN.

The investigation and its publication were supported by grants from Kristianstads läns landsting, Statens medicinska forskningsråd, Nordisk insulinfond and *Svenska diabetesförbundet*.

Any attempt to give a detailed survey of the voluminous literature on the aspects pertinent to the present investigation would be futile. It was therefore decided to limit references to a reasonable minimum and to draw generously on recent Scandinavian monographs on diabetic epidemiology and *other related topics* by LUNDBAEK (1953), LARSETH (1953), ENGLESON (1954), KORNERUP (1955), SKOUBY (1956), FAGERBERG (1959) and THOMSEN (1965).

II. Clinical material

The material comprised 598 diabetics (282 males and 316 females) who had been seen at the department of medicine, Central hospital, Kristianstad, between September 1963 and January 1966 and who were living in the hospital catchment area which had a population of 95,717 inhabitants on Dec. 31, 1965. The material was supplemented by inquiries among health officers and practitioners in the area and by new cases of disease demonstrated in association with a general health survey carried out between 1963 and 1966. The supplementary cases detected represented about 10 % of the entire material.

Diabetes mellitus was said to be present only if the fasting blood sugar had been more than 130 mg/100 ml on at least 3 occasions. Thus temporary elevation of the blood sugar level in association with myocardial infarction, cerebral insult, liver disease, pregnancy or during treatment with chlorthiazide preparations were not by themselves sufficient to warrant a diagnosis of diabetes mellitus. Nor was the occurrence of a so called diabetic glucose tolerance test.

The sample studied comprised persons from the country and from small and medium sized towns and though it included none from large towns it may be regarded as representative of the Swedish population (NILSSON et al. 1964). The therapeutic regimen applied at the hospital includes instructions to restrict consumption of sugar, bread, potatoes and other readily absorbed carbohydrates as well as fat. In recent years increased physical exercise has been recommended.

Different types of insulin have been used, often in combination and in repeated doses per day. Oral treatment was used particularly for middle aged and elderly diabetics and was generally replaced by insulin if the amount of urinary sugar per 24 hours exceeded 15 g. During the 1955—1960 period carbutamide and tolbutamide were the most widely used preparations and in recent years chlorpropamide. Phenformin has been employed only to a small extent.

For several decades special interest has been focused on diabetes at the department (SILWER 1958). This might have resulted in an unusually close observation of such patients. On comparison with the SILWER's figures from 1954 for the entire district of Kristianstad with 258 000 inhabitants of which the present population represents 37.1 % the frequency has increased, except for middle aged women. This increase might however be ascribable partly to more extensive health control in the district.

The ratio of males to females during 12 years between SILWER's investigation and the present study increased from 0.75 to 0.89 which is comparable to that found in England by MALINS et al. (1965) who reported an increase

Table 1 *Population studied and series A and B*
Percentages of persons who were examined roentgenologically and ophthalmologically
Comparison with figures given by SILWER (1958) for entire district of Kristianstad in 1954 which then had a population of 258 000 of which the present investigation represents 37 1 %

Sex	Age (years)	Total recorded	Examined rtg (%)	Examined ophthalm (%)	Number of diabetics in relation to Silwer's series (%)	Series A	Series B
males	60—79	130	83 1	76 9	134.8	40	40
	40—59	102	83.3	78 4	150 1	40	40
	20—39	50	76 0	76 0	151.5	20	22
females	60—79	200	84 0	81 0	135.3	40	40
	40—59	83	91 6	91 6	112 9	30	30
	20—39	33	63 6	93 9	139 1	13	19
Total		598	80.3	83 0	137.3		

from 0 62 to 0 90 — likewise due mainly to decreased frequency of the disease in middle aged women

To enable direct comparison independent of age between controls studied previously in the area (NILSSON et al 1964) and diabetics who had had the disease for a long respectively short time in the present population two series A and B were constructed The man and the woman with the shortest duration of the disease (but at most 5 years) in each age class were assigned to series A and those with the longest duration (but at least 7 years) were allotted to series B The average duration of the disease was 1.5 years in series A and 20 years in series B When there were several persons who had had the disease for the same duration the one who was born earliest in the month of birth was chosen If necessary the group was supplemented with patients from the next two age classes above or below, the patient whose date of birth was nearest the ages in question being chosen

The members of series A and B were also distributed among the age groups 20—39 40—59 and 60—79 years

It is clear from Table 1 that the lowest age groups in series A and B were not complete But the selection was made in such a way that as far as age is concerned they may be regarded as representative In all comparisons between the values obtained in the diabetic males or females and the control values the mean values of the mean found in the subgroups were used This was done in order to avoid any influence of the variation in the size of the subgroups

All the patients were examined at the departments of medicine roentgenology and ophthalmology of the hospital The frequencies of these examinations are given in Table 1

III. Methods

Since the beginning of the investigation in September 1963 the diabetics were reviewed annually, the review including physical examination and laboratory studies As a rule the review was carried out in association with routine follow-up at the outpatient department Except for the roentgenologic and ophthalmologic data those given in what follows are the means of values noted at two to three examinations The examinations of the blood chemistry were performed on samples collected in the morning with the patient in the fasting state

ANTHROPOMETRIC METHODS

Since the examination was done at the outpatient department the patients were not weighed naked, but simply with their shoes and jackets off The weights of the clothes in a control series of 40 persons were found to vary between 0 8 and 1 5 kg In the control series there was no systematic deviation of this weight and therefore the average bodyweight in the present material was reduced by 1 2 kg

In the analysis of the bodybuild the following factors were determined

Skeletal length factor expressed as bodyheight without shoes

Skeletal sturdiness factor expressed as femoral condylar breadth and bistyloid radio ulnar breadth, measured according to MARTIN (1928) and LINDEGÅRD (1953, 1956) This sturdiness factor is highly correlated with muscle mass (LINDEGÅRD 1953, 1956)

Fat-free bodyweight calculated according to v DÖBELN (1959, 1961) and according to the formula FFW = $= 15.1 \, (H^2 \; F \; R \; 100)^{0.712}$ (FFW is fatfree weight, H height, F sum of right and left femoral condylar breadths and R sum of right and left radio ulnar bistyloid breadths FFW is given in kilograms and H, F and R in metres)

Weight of bodyfat assessed by the difference between total bodyweight and fatfree bodyweight, calculated as above Bodyfat has been expressed as per cent of total bodyweight

The values of fatfree weight and bodyfat obtained by these methods agree with those reported by BROZEK et al (1953) and YOUNG et al (1963) who used the densitometric technique, and with those of DISSMAN et al (1964) who derived the values by examining fluid compartments The body fat is lower throughout than the values obtained in isotope studies by ANDERSSON (1963), MCNEELY et al (1963) and STEINKAMP et al (1965) This may be due to a larger mean amount of bodyfat in North America (see v DÖBELN 1961)

OTHER PHYSICAL EXAMINATIONS

The *pulse frequency* was calculated from the LCG recording During the LCG recording the patient has been recumbent

The *blood pressure* was measured after the subject had been lying for 3 minutes A mercury manometer of type ERKA was used The diastolic pressure was registered as the pressure at with the sounds disappeared

Roentgenography of lower limbs Radiographs were taken of the soft tissue with a focus film distance of about 100 cm primary diaphragm and Potter Bucky diaphragm Two views one at right angles to the other were taken of the thigh The frontal view of the lower leg was taken with *the leg turned slightly inward to* enable estimation of the space between the tibia and the fibula while the lateral view was taken with the leg in normal position The popliteal region was inspected either in a lateral view taken over the thigh or in a lateral view of the lower leg The foot arteries were judged in frontal and oblique views taken mainly over the area of the metatarseal bones and the area around the malleoli The following grades of calcifications were distinguished

0 No calcifications
1 Minimal calcifications
2 Moderate calcifications
3 Marked calcifications

Such grading has been used previously in similar investigations *by e g* LANSBURY & BROWN (1934), KEIDING et al (1952) and HAIMOVICI et al (1960)

The thigh, the lower leg and the foot were judged separately Values obtained in the various areas were taken together after which the following *classification was set up*

0 0—0—0
1 1—0—0, 1—1—0
2 1—1—1, 2—0—0, 2—1—0
2—2—0
3 2—1—1 2—2—1 3—0—0
3—1—0
4 3—1—1 3—2—0 3—2—1
2—2—2
5 3—2—2 3—3—1 3—3—2
6 3—3—3

All of the roentgenograms were judged by one of us (FROSTBERG) who had also judged the films in the previous investigation of the normal population (NILSSON et al 1964)

The arterial pulsations on the feet were palpated bilaterally *in the arteria dorsalis pedis* and arteria tibialis *posterior* The patients were then distributed among five classes according *to the absence* of palpable pulsations in one or more areas examined

Appreciation of vibration was tested by applying a tuning fork (C^{128}) to the medial and lateral malleoli on both sides

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Since the beginning of the investigation in September 1963 the diabetics were reviewed annually, the review including physical examination and laboratory studies. As a rule the review was carried out in association with routine follow-up at the outpatient department. Except for the roentgenologic and ophthalmologic data those given in what follows are the means of values noted at two to three examinations. The examinations of the blood chemistry were performed on samples collected in the morning with the patient in the fasting state.

ANTHROPOMETRIC METHODS

Since the examination was done at the outpatient department the patients were not weighed naked, but simply with their shoes and jackets off. The weights of the clothes in a control series of 40 persons were found to vary between 0.8 and 1.5 kg. In the control series there was no systematic deviation of this weight and therefore the average bodyweight in the present material was reduced by 1.2 kg.

In the analysis of the bodybuild the following factors were determined:

Skeletal length factor expressed as bodyheight without shoes.

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OTHER PHYSICAL EXAMINATIONS

The *pulse frequency* was calculated from the ECG recording During the ECG recording the patient has been recumbent

The *blood pressure* was measured after the subject had been lying for 3 minutes A *mercury manometer* of type ERKA was used The diastolic pressure was registered as the pressure at with the sounds disappeared

Roentgenography of lower limbs Radiographs were taken of the soft tissue with a focus film distance of about *100* cm primary diaphragm and Potter Bucky diaphragm Two views one at right angles to the other were taken of the thigh The frontal view of the lower leg was taken with the leg turned slightly inward to enable estimation of the space between the tibia and the fibula while the lateral view was taken with the leg in normal position The popliteal region was inspected either in a lateral view taken over the thigh or in a lateral view of the lower leg The foot arteries were judged *in frontal* and oblique views taken mainly over the area of the metatarseal bones and the area around the malleoli The following grades of calcifications were distinguished

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The thigh the lower leg and the foot were judged separately Values obtained in the various areas were taken together after which the following classification was set up

0 0—0—0
1 *1—0—0 1—1—0*
2 1—1—1 2—0—0 2—1—0 2—2—0
3 *3—1—1* 2—2—1 3—0—0 3—1—0
4 3—1—1 3—2—0 3—2—1 2—2—2
5 3—2—2 3—3—1 3—3—2
6 3—3—3

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The *arterial pulsations on the feet* were palpated bilaterally in the arteria *dorsalis pedis* and *arteria tibialis* posterior The patients were then distributed among five classes according to the absence of palpable pulsations in one or more areas examined

Appreciation of vibration was tested by applying a tuning fork (C^{128}) to the medial and lateral malleoli on both sides

Changes in the ocular fundi in diabetes mellitus were graded according to the following criteria

- Group 1 scattered microaneurysms
- Group 2 microaneurysms and retinal hæmorrhages
- Group 3 as in group 2 plus exudative foci
- Group 4 as in group 3 plus proliferative changes in the retina and/or vitreous body

The *hypertensive vascular changes* in the ocular fundi were judged according to KEITH et al (1939)

- Group 1 slight spasm of vessels
- Group 2 increased spasm, increased calibre and arterio venous crossing phenomenon
- Group 3 retinal hæmorrhages and exudative foci
- Group 4 widespread hæmorrhages and exudates as well as papillœdema

The *degree of cataract* was estimated with the aid of a corneal microscope, and in transmitted light with an ophthalmoscope The following grades were distinguished

- 0 no cataract
- 1 peripheral cataract not affecting vision
- 2 moderate cataract
- 3 severe cataract

The *intraocular pressure* was measured with a SCHIÖTZ tonometer One and the same instrument was used throughout and the 0 position of the tonometer was checked every day The measurements were made as far as possible at the same time of the day The subjects were examined in the supine position

The degree of *arcus lipoides corneæ* (a l c) was estimated by examination with a corneal microscope Four degrees were recognized

LABORATORY METHODS

The *blood sugar* up to 1960 was determined according to HAGEDORN JENSEN and afterwards by the ortho toluoidine method of HULTMAN (1959) Capillary samples were used With but few exceptions the examinations were carried out in the morning with the patient in the fasting state

Urinary sugar was determined according to Benedict, and from 1960 according to HULTMAN (1959), and is expressed in grams/24 hours

Tendency to acidosis was judged from the acetone bodies measured by the method of Gerhard and from the acetic acid measured *ad modum* Legal In recent years Acetest "Ames" has also been used in screening examinations

The patients were classified according to the following criteria

1. absence of signs of acidosis at all examinations
2. positive reactions occasionally
3. regularly positive reactions
4. occurrence of diabetic coma or precoma

Cholesterol in the serum was determined with LIEBERMANN BURCHARD s reaction according to CRAMER & ISAKSSON (1959) who have shown this method to give results in good agreement with those obtained by the method of SCHOENHEIMER & SPERRY (1934)

The *serum glutamic pyruvic transaminase (GPT)* was determined according to REITMAN & FRANKEL (1957) and Sigma (1960)

The *creatinine* was determined according to LOCKEN (1954)

ESBACH's method was used for quantitative determination of *proteinuria* The determinations were made on 24 hour urine samples

Microscopical examination of urine Newly voided urine was centrifuged for about 5 minutes and the sediment was examined (X450) unstained The results *i e* number of leukocytes as well as erythrocytes were classified as follows

1 0—1 cells/field
2 2—10 cells/field
3 more than 10 cells/field

THERAPY AND CONTROL

In the description of severity and regulation of the disease the following methods were used

Therapy is described mainly as the insulin dose required The following classes were used

1 dietary measures only
2 oral therapy
3 less than 20 I U insulin/24 hours
4—11 20—29 I U 30—39 I U/24 hours etc
12—13 100—119 I U, 120—139 I U/24 hours
14 140—169 I U/24 hours
15 At least 170 I U/24 hours

Therapy and regulation were judged on the basis of a scrupulous calculation of the mean values of values noted at the examinations in the course of a 2 year period If the number of values was less than 5 it was supplemented by the value or values noted closest in time but only during the previous or following year If the patient had been admitted to hospital the values on the first day and the last day in hospital were included in the calculations because the sugar values on admission were usually higher and on discharge lower than on the average As the examination period we used years 1—2 5—6 etc with 5 years interval up to a duration of 30—31 years As a collective expression of these values the mean of the values obtained at 5 year intervals was also calculated in the aforementioned way

An expression of the *stability* of the regulation of the patients was obtained by calculating the average deviation of the urinary sugar and blood sugar from the mean value of the above—mentioned examinations

Follow-up is given as the number of examinations per year Admission to hospital was counted as 2 examinations The examinations were usually performed at 2—5 month intervals

GENETIC RISK

The risk of being a carrier of an inherited factor increasing the penetrance of diabetes mellitus was calculated from the known familial occurrence of diabetes The following classes were constructed

0 Diabetes not known in family
1 Diabetes known in grand parent, uncle or aunt
2 Diabetes known in parent, child, or grand parents on same side
3 Diabetes known in sib or grandparents on different sides
4 Diabetes known in one parent, and grandparent or parent's sib of other side

STATISTICAL METHODS

Arithmetic means, standard deviations (S D) and coefficients of correlation (r) were calculated according to conventional statistical methods

In the evaluation of correlation coefficients the concordance and structure of the correlations must be taken into account Therefore no degree of significance are given in the tables In the evaluation of the probability of a single correlation to differ from zero the following criteria of significance for different pairs of observations were used in the present investigation

Pairs of observations	Level of significance 0 1	0 05	0 01	0 001
35	0 27	0 32	0 42	0 52
100	0 16	0 19	0 25	0 32
200	0 12	0 14	0 18	0 23
300	0 10	0 11	0 15	0 19
500	0 07	0 09	0 12	0 15

IV Treatment and control

To facilitate description of the treatment the patients were grouped in the way described under Methods

Table 2 shows that the insulin requirements increased markedly after the patients had had diabetes for 5—6 years to culminate after 15—16 years when the average insulin requirement was about 55 I U./24 hours Of 88 diabetics 12 (13 6 %) required more than 80 I U./24 hours insulin at this time and 7 (8 0 %) required oral treatment or, in some cases only dietary restrictions The highest insulin requirement recorded was 180 I U./24 hours

Table 2 *Class of therapy (see Methods) according to sex and patients' ages as well as duration of diabetes*

Duration	Age / Sex	20—39	40—59	60—79	total
1—2 years	M	5 1	3 8	2 8	3 9
	F	5 0	3 5	2 8	3 8
	M+F				3 8
5—6	M	6 9	5 6	4.0	5 5
	F	6 9	4 6	4 6	5 4
	M+F				5 6
10—11	M	8 1	5 9	4 8	6 3
	F	8 5	5 8	5 1	6.5
	M+F				6 4
15—16	M	7 7	7 2	6 0	7 0
	F	8 4	6.5	6 0	7 0
	M+F				7 0
20—21	M+F				6 4
25—26	M+F				6 6
30—31	M+F				6 4
Mean therapy	M	6 1	4 4	3 4	4 6
	F	6.3	4 2	4 0	4 8
	M+F	6 2	4.3	3 7	4 7

The frequencies of persons treated only with dietary restrictions or dietary restrictions and tablets (as a rule, chlorpropamide) are given in Table 3 During the first 2 years of treatment such therapy was sufficient for two thirds of those in the age group 60—79 years and for half of those in the 40—59 year group Women were slightly preponderant in these groups About 40 % of the el*derly men and the elderly and middle-*aged women were still able to manage on such treatment after 5 years

To assess regulation the average values found for the excretion of urine sugar per 24 hours fasting blood sugar and fluctuations of the urine sugar and fasting blood sugar about these mean values were calculated in the way given under "Methods" It is clear from Table 4 that the mean *values invariably tended to fall with* advancing age, the fall being most marked between the 20—39 and 40—59 year age groups Only regarding the urine sugar level was any difference found with sex The lower values found for females is hardly due to a decrease of the daily urine output but rather to a considerable increase in the number of women with only slight or no glucosuria, especially in the 40—59 year group — see Table 5 The finding can be compared with the decreased frequency of glu*cosuria noted in the women in the* glucose tolerance test (NILSSON et al 1964)

Table 6 shows that the urine sugar levels reached a maximum after the patients had had the disease for 15—16 years while the blood sugar level continued to increase during the following 5 year period The stability of the regulation as reflected by the fluctuation of the blood sugar level *showed a slow but continuous decrease* throughout the observation period

Table 3 *Frequency (%) of diabetics who had had the disease for different periods and who were treated by dietary methods or who received oral treatment*

Duration / Age sex	1—2 years		5—6 years	
	diet	tabl	diet	tabl
60—79				
males	28 3	35 5	19 1	23 5
females	23 4	42 5	18 5	22 2
40—59				
males	15 5	27 8	5 2	10 3
females	16 2	35 3	3 4	37 9
20—39				
males	5 3	15 8	4 0	—
females	9 5	19 0	8 0	—

Table 4 *Levels and fluctuation of blood and urine sugar in males and females grouped according to age*

Age	Sex	Urine sugar (g/24 h) mean	Urine sugar (g/24 h) SD	Blood sugar (mg/100 ml) mean	Blood sugar (mg/100 ml) SD	Fluctuation urine sugar mean	Fluctuation urine sugar SD	Fluctuation blood sugar mean	Fluctuation blood sugar SD
60—69									
	males	13.6	13.2	156	34	10.1	7.6	33	16
	females	9.2	11.6	160	35	8.2	7.0	35	17
40—59									
	males	16.0	11.3	159	29	12.2	7.1	38	13
	females	12.7	13.0	159	30	10.4	8.0	35	17
20—39									
	males	23.2	12.8	167	38	16.1	8.0	46	15
	females	19.6	12.9	177	42	14.4	7.8	48	19
total									
	males	17.6	12.4	161	34	12.8	7.6	39	15
	females	13.8	12.5	165	38	11.0	7.6	39	18

Table 5 *Frequency (%) of urine sugar of at most 1 g a day in males and females grouped according to age*

Sex \ Age	60—69	40—59	20—39
males	14.1	5.9	4.1
females	20.5	24.6	6.1

Table 6 *Mean levels and fluctuation of urine sugar and blood sugar related to duration of diabetes*

Duration (years)	Urine sugar (g/day)	Blood sugar (mg/100 ml)	Fluctuation urine sugar	Fluctuation blood sugar
1—2	11.5	157	9.3	36
5—6	17.4	163	12.7	41
10—11	18.0	162	14.6	40
15—16	19.5	162	12.6	43
20—21	16.7	179	10.5	47
25—26	1[illegible].4	169	10.7	49
30—31	15.3	156	10.0	49

Table 7 *Coefficients of correlation between therapy, follow up and various expressions of diabetic control*

	Therapy		Follow up		Urine sugar		Blood sugar		Fluctuation of urine sugar		Fluctuation of blood sugar	
	M	F	M	F	M	F	M	F	M	F	M	F
Follow up	17	41										
Urine sugar	51	61	16	36								
Blood sugar	38	51	10	28	53	67						
Fluctuation of urine sugar	38	53	23	41	82	86	41	55				
Fluctuation of *blood sugar*	*54*	*50*	*20*	*28*	*41*	*48*	*46*	*55*	*42*	53		
Acidity	39	41	09	34	21	36	16	28	13	29	27	41

The relationship between treatment, intensity of follow up and various expressions of disturbances of the glucose metabolism are given in Table 7 which is calculated on the average values noted in the patients during the entire period of their disease It was found that treatment, *i e* mainly the amount of insulin, in the group examined was based chiefly on the amount of urine sugar and the fluctuations of the blood sugar level The intensity of follow up, *i e* intervals between consecutive examinations, appeared to vary especially with the fluctuation of the sugar level The fluctuation of the urine sugar was more dependent on the urine sugar level than was the blood sugar fluctuation on the blood sugar level As to acidosis the highest correlation found was that with the fluctuation of the blood sugar level

V Bodyweight and other anthropometric data

Table 8 shows that the average body weight of the diabetics did not differ significantly from normal. This holds not only for series A and B but also for the entire diabetic series uncorrected for age where the average over weight compared with the bodyweight of normals from the same district was 1.0 kg for men and 1.3 kg for women.

Comparison between those who had the disease for a short time and those who had it for a long time revealed a distinct difference between the groups with a clear overweight for both the men and the women who had had the disease for a short time — especially in the age group 40—59 years. In those who had had diabetes for a long time the values were decreased in all ages in both the men and the women.

The overweight in recently discov

Table 8 *Anthropometric findings in diabetes of short (A) and long (B) duration compared with normal values (N)*

			Weight (kg)		Height (cm)		Per cent bodyfat		Fatfree weight (kg)		Condylar breadth (cm)		Bistyloid breadth (cm)	
			mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
Males	total	N	75.1	9.9	173.2	5.1	20.2	10.1	59.1	4.7	9.60	0.38	6.00	0.27
		A	78.0	12.7	174.1	6.1	20.3	11.6	61.5	5.9	9.90	0.46	6.10	0.32
		B	72.8	9.3	173.9	6.0	15.1	10.2	60.3	6.0	9.80	0.43	5.98	0.36
	20—39 years	N	71.9	9.4	175.4	8.4	16.1	8.4	59.7	4.9	9.60	0.39	5.87	0.26
		A	73.0	12.2	176.0	5.9	16.5	10.4	61.3	5.7	9.88	0.52	5.98	0.31
		B	66.8	7.9	174.9	6.0	10.9	8.1	59.4	6.2	9.79	0.40	5.85	0.34
	40—59 years	N	76.2	9.5	173.1	5.0	21.3	10.8	59.2	4.6	9.56	0.37	6.00	0.26
		A	83.4	14.9	174.4	6.1	23.2	13.8	63.0	5.6	9.92	0.39	6.16	0.32
		B	71.3	9.2	174.0	5.7	15.4	11.1	61.2	5.3	9.[illegible]9	0.38	6.06	0.41
	60—79 years	N	76.6	10.3	171.4	4.6	22.5	9.6	58.4	4.5	9.64	0.36	5.94	0.29
		A	77.6	11.0	171.9	6.3	20.0	10.6	60.8	6.4	9.89	0.48	6.15	0.32
		B	75.5	10.7	172.8	6.2	19.0	11.4	59.9	6.4	9.84	0.52	6.03	0.34
Females	total	N	6[illegible].1	11.6	162.4	5.7	30.1	10.0	46.0	4.2	8.85	0.48	5.22	0.28
		A	69.7	16.0	162.2	4.9	31.5	12.8	47.3	5.7	9.23	0.59	5.19	0.29
		B	63.9	10.3	161.4	6.4	27.4	10.1	46.1	4.6	8.92	0.49	5.70	0.26
	20—39 years	N	64.3	10.6	163.4	5.0	27.5	9.5	46.0	4.3	8.81	0.39	5.15	0.29
		A	63.9	16.0	164.9	4.5	31.7	14.6	45.9	5.2	8.90	0.42	5.03	0.29
		B	61.8	7.2	162.2	7.4	26.3	7.4	45.6	4.2	8.88	0.46	5.06	0.22
	40—59 years	N	67.1	11.9	161.8	5.5	30.8	10.6	45.1	4.0	8.[illegible]3	0.38	5.16	0.23
		A	77.5	20.2	162.5	5.1	34.6	13.2	49.2	6.8	9.19	0.53	5.31	0.28
		B	61.9	11.2	161.1	5.5	26.5	10.7	45.3	4.6	8.75	0.51	5.17	0.27
	60—79 years	N	70.5	11.6	162.0	6.7	32.8	9.0	47.0	4.2	9.04	0.61	5.24	0.30
		A	67.7	11.8	159.3	5.2	28.3	10.6	46.8	5.1	9.40	0.81	5.22	0.31
		B	67.9	12.4	160.0	6.2	29.2	12.1	4[illegible].3	4.8	9.13	0.51	5.20	0.28

ered cases of diabetes seems to be due to an increase of fatfree weight as well as to an increase of fat A definite increase of bodyfat was thus noted only in young and middle aged women, while the fatfree weight, calculated according to v DÖBELN, was invariably increased among the males but less so in the females

Increase of fatfree weight was not due to increase in height but to an increase of the sturdiness factor described by measurements of the diameters of certain skeletal parts The large weight difference between diabetics who had had the disease for a long time and those who had had it for only a short time was due above all to a marked reduction of the fat factor Also the fatfree weight invariably decreased but to a lesser degree with increasing duration of the disease

The anthropometric technique used showed a positive relation between the 'sturdiness'-factor, expressed as the breadth of the condyle, and the amount of body fat — owing to the negative correlation between bodyheight and bodyfat, however no correlation was found between fatfree weight and bodyfat

The changes observed in the diabetic adults corresponded to those noted with the same technique in young diabetic men (NILSSON 1962) It appears to be established that diabetes is associated with an increase of the fatfree weight — and then particularly of the sturdiness factor described by LINDEGÅRD which corresponds to SHELDON's mesomorphy Whether this is due to a true genetic linkage or whether the factors causing the increased sturdiness also increase the risk of penetrance of diabetes is debatable It is, however, remarkable that overweight occurrs also in healthy relatives of diabetics (NILSSON 1962, 1964)

Anamnestic data of the patients before the discovery of the disease were not available in the present material and it cannot be excluded that in many cases the onset had been preceded by an increase in bodyweight which might have decreased in association with the appearance of the disease At the examination of the 46 men and 36 women whose diabetes had been discovered during the last year bodyweight for each sex was 1 0 kg less than the corresponding values in series A The bodyfat was also decreased at such an examination

The low values found for the fat factor in the diabetics in the present series may have been due to a relatively strict dietary regimen The examination however probably shows that the overweight so often reported in diabetics series is due to an equal extent to an increase of fatfree weight as to an increase of the bodyfat Previously reported series of adult diabetics are difficult to evaluate — with but few exceptions (PYKE & PLEASE 1957) — because the controls were inadequate and because the duration of the disease was not taken into account in the analysis of the data obtained

VI Interrelationship between various diabetic complications and their variation with the duration of diabetes

The correlation between two symptoms — *in this case* metabolism disturbed by diabetes and various so called diabetic complications — may be due to

1 the same endogenous precipitating factors in both
2 the same exogenous precipitating factors in both
3 the existence of one symptom favouring the development of the other

In this evaluation the influence of isolates and the effect of reproduction are not taken into account

In alternative 1 one would expect an increased frequency of complications among relatives of diabetics in alternative 2 also an increase in frequency of such complications among persons living under similar conditions —*e g* married couples living together and in alternative 3 a correlation between the duration of the precipitating factor and the intensity of the secondary symptom

Alternative 1 was evaluated from correlations between the frequency of various complications and the occurrence of diabetes in close relatives which here may be regarded as an expression of the degree of factors increasing the risk of penetrance No such correlation was found except for a somewhat higher frequency of retinopathy in persons with an increased familial occurrence of diabetes — for further discussion see Chapter VII

Alternative 2 could not be judged because the occurrence of diabetes among married men was so low that analysis would have required a much larger series

Alternative 3 appears the most probable one and it was therefore of interest to note that retinopathy was the complication that increased most in frequency with increasing duration of diabetes (Table 9) Another clearly positive correlation was found between the roentgenologically demonstrated calcifications in the arteries of the lower limbs Less pronounced positive correlations between different complications and the duration of the disease are analysed further in Chapter VIII A noteworthy negative correlation with GPT was invariably found

Table 10 shows a high correlation between the vascular changes and neurological signs in the lower limbs a finding reported previously by FAGERBERG (1959) for example Their correlation with retinopathy was less

ered cases of diabetes seems to be due to an increase of fatfree weight as well as to an increase of fat. A definite increase of bodyfat was thus noted only in young and middle aged women, while the fatfree weight, calculated according to v. DOBELN, was invariably increased among the males but less so in the females.

Increase of fatfree weight was not due to increase in height but to an increase of the sturdiness factor described by measurements of the diameters of certain skeletal parts. The large weight difference between diabetics who had had the disease for a long time and those who had had it for only a short time was due above all to a marked reduction of the fat factor. Also the fatfree weight invariably decreased but to a lesser degree with increasing duration of the disease.

The anthropometric technique used showed a positive relation between the "sturdiness" factor, expressed as the breadth of the condyle, and the amount of body fat — owing to the negative correlation between bodyheight and bodyfat, however, no correlation was found between fatfree weight and bodyfat.

The changes observed in the diabetic adults corresponded to those noted with the same technique in young diabetic men (NILSSON 1962). It appears to be established that diabetes is associated with an increase of the fatfree weight — and then particularly of the sturdiness factor described by LINDEGÅRD, which corresponds to SHELDON's mesomorphy. Whether this is due to a true genetic linkage or whether the factors causing the increased sturdiness also increase the risk of penetrance of diabetes, is debatable. It is, however, remarkable that overweight occurrs also in healthy relatives of diabetics (NILSSON 1962, 1964).

Anamnestic data of the patients before the discovery of the disease were not available in the present material and it cannot be excluded that in many cases the onset had been preceded by an increase in bodyweight which might have decreased in association with the appearance of the disease. At the examination of the 46 men and 36 women whose diabetes had been discovered during the last year bodyweight for each sex was 1.0 kg less than the corresponding values in series A. The bodyfat was also decreased at such an examination.

The low values found for the fat factor in the diabetics in the present series may have been due to a relatively strict dietary regimen. The examination however, probably shows that the overweight so often reported in diabetics series is due to an equal extent to an increase of fatfree weight as to an increase of the bodyfat. Previously reported series of adult diabetics are difficult to evaluate — with but few exceptions (PYKE & PLEASE 1957) — because the controls were inadequate and because the duration of the disease was not taken into account in the analysis of the data obtained.

Table 10 *Coefficients of correlation between different types of diabetic complications*

		Pulsat feet		Calcificat lower extr		Vibr perception		Achilles jerk		Creatinin/s		Proteinuria		Vision		Cataract		Re pa
		M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M
Total	Pulsations feet			—.6	—26	.35	.37	.23	22	—13	—17	—10	—10	.08	.22	—.22	—21	—08
60–79				—11	—15	27	.33	.24	15	—06	—13	—16	—12	.05	12	—.19	—11	—18
40–59				—16	—30	07	20	13	.26	—04	—08	—10	—04	—.04	.36	10	—24	—.02
20–39	Calcificat. lower limb	—.6	—26			—31	—22	—.21	—27	06	—02	11	09	[illegible]	—.31	.39	.23	16
						—08	—09	—.02	—20	—12	—14	11	06	—12	—18	.20	09	17
						—08	—.37	—18	—20	—06	—06	05	—02	—42	—.28	16	.22	44
	Vibr percept	.35	.37	—31	—22			.38	.33	—23	—18	—.03	—07	.24	.24	—.29	—22	.05
								35	24	—14	—13	—12	—.09	19	15	—11	—14	—01
								23	.33	—03	—02	07	05	12	15	—.08	—04	12
										—11	—24							
	Achill jerk	23	.22	—.21	[illegible]	.38	.33			—15	—08	—12	—04	.25	[illegible]8	—.36	—.21	—1[illegible]
										—1	—.09	—13	05	.23	.20	—.31	—11	—0[illegible]
										—.06	—.22	—18	—16	.13	.42	—.35	—.29	—16
	Serum creatinin	—13	—17	06	—02	—22	—18	—15	—08			38	26	—.30	—18	.23	.20	.2[illegible]
												.35	.31	—43	—11	.20	14	.2[illegible]
												40	16	—01	—.31	09	.25	.0[illegible]
												.51	07					
	Proteinuria	—10	—10	11	.09	—03	—07	—12	—04	.38	26			—.20	—14	02	06	4[illegible]
														—.24	—05	—07	03	.2[illegible]
														—.23	—35	15	.05	.3[illegible]
																		4[illegible]
	Vision	08	.22	[illegible]	—.31	.24	.24	.25	—.8	—.0	18	—.20	—14			—45	—.8	—.2[illegible]
																—.28	—49	—.2[illegible]
																—.35	—.09	—.3[illegible]
																[illegible]8	—83	[illegible]
	Cataract	—23	—21	.39	.23	—29	—22	—.36	—.1	23	.20	02	06	—45	—.58			1[illegible]
																		1[illegible]
																		1[illegible]
																		.1[illegible]
	Retinopathy	—.08	—.22	16	19	05	—18	—10	—24	22	09	40	[illegible]4	—26	—4[illegible]	10	.29	

striking but distinct, especially concerning the roentgenographic vascular changes The correlation between the above mentioned symptoms and cataract was partly due to both symptoms being correlated with the patient's age Retinopathy was found to be correlated with both vascular changes and proteinuria but no definite correlation was found between the two last mentioned complications

Retinopathy is not dependent on age so that age may be ignored in the investigation of the correlation between retinopathy and other symptoms

Impaired vision in diabetics was generally found to be due to a larger extent to cataract than to retinopathy

Table 9 *Coefficients of correlation between different diabetic complications and age, duration of the disease and degree of occurrence of familial diabetes*

		Age	Age at manifest	Duration	Therapy	Follow up	Urine sugar	Blood sugar	Fluct urine sugar	Fluct blood sugar	Acidose tendency	Famil d m occurr
Retinopathy	M	07	−29	51	33	−03	19	28	05	29	14	11
	F	04	−14	42	25	02	16	14	06	10	05	05
Cataract	M	43	34	05	−15	−07	−06	−03	−13	−06	−09	−10
	F	34	22	18	11	00	−02	13	−08	−06	−02	−06
Ocular tonus	M	−05	06	06	−03	08	−03	00	−04	02	−04	02
	F	04	00	07	09	09	07	12	06	05	−06	−07
Arcus corneae	M	51	−37	09	−11	−16	−11	−03	−15	−23	−17	−03
	F	40	37	−15	−13	−03	02	04	04	−05	−09	−03
Vision	M	−22	−15	−09	08	04	07	07	14	−03	09	−01
	F	−27	−20	−15	−11	04	02	06	06	−06	03	20
Proteinuria	M	−02	−10	18	04	−06	−03	10	−05	14	11	05
	F	05	04	−01	02	09	−04	05	−03	02	−04	00
Serum creatinin	M	23	19	00	−16	−11	−29	−11	−29	−11	−13	−10
	F	22	18	01	−08	−11	−24	−10	−24	02	−09	−10
Hematuria	M	06	07	−01	−02	00	−02	15	−04	10	06	11
	F	01	−01	05	08	−02	03	12	02	07	01	01
Pyuria	M	14	17	−09	−11	−09	−03	06	−03	−05	−04	04
	F	10	09	−03	−02	−04	−10	−04	−07	02	−02	04
Pulsations feet	M	−41	−31	−09	04	07	11	08	12	10	14	02
	F	−26	−18	−05	−11	−01	02	−10	04	−09	04	−01
Calcifications lower extr	M	59	39	19	−10	−10	−10	03	−19	−03	−14	06
	F	43	24	15	04	−05	−08	−04	−14	05	00	−03
Vibration percept	M	−46	−39	01	07	18	05	−05	10	07	11	06
	F	−35	−24	−09	−06	03	03	−14	05	−03	08	11
Achilles jerk	M	−31	−22	−09	−06	09	05	−03	12	−02	−02	02
	F	−31	−19	−08	00	−04	−01	−06	05	05	03	10
Systolic blood pr	M	43	37	−04	−19	−13	−18	07	−22	−07	−15	02
	F	49	44	−10	−09	−03	−19	−08	−17	−15	−19	−04
Serum cholesterol	M	04	03	00	−04	00	−01	12	−07	−07	01	06
	F	13	08	05	−05	−07	−04	02	−02	01	−08	02
Serum GPT	M	−08	−07	−30	−15	−03	−19	−07	−00	−06	−04	−05
	F	−02	12	−27	−11	−03	−06	−10	−02	−07	−00	09

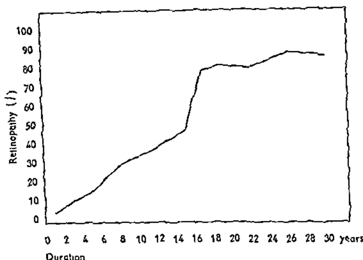


Fig 1 *Frequency of retinopathy in relation to duration of diabetes (males+females)*

dependent mainly on the duration of the disease Table 13 was constructed to show the increasing frequency of retinopathy with the duration of diabetes Fig 1 shows that after 16 years duration of the disease the frequency of retinopathy was fairly steady and then 80—90 % of the patients had the complication The curve in Fig 1 agrees very well with that given by COLWELL (1966) which is based on a compilation of several larger investigations The values are also of the same order as after a corresponding duration in Scandinavian studies by LARSSON et al (1952) LUNDBAEK (1953) ENGLESON (1954) and KORNERUP (1955) but they are higher than what was found by BARTELS & POULSEN (1950) and MÅRTENSSON & PALM (1950) This discrepancy may perhaps be due to differences in the composition of the series but probably also to the influence of the strict dietary restrictions prescribed by the classical diabetic regimen and the shortage of food during the last war

Table 13 *Frequency (%) of retinopathy in different age classes*

Duration (years) / Age	Males			Females			Males+Females		
	—7	8—15	16—	—7	8—15	16—	—7	8—15	16—
Age 20—39	6.3	35.7	100.0	16.7	16.7	81.8	11.5	26.2	90.9
40—59	5.1	38.1	90.0	12.8	29.4	95.0	9.0	33.8	92.5
60—79	8.8	31.8	61.1	20.0	53.8	90.5	14.4	42.8	75.8
total	7.3	35.1	81.3	17.0	41.7	86.3	12.2	39.9	83.8

VII. The development of diabetic retinopathy

As previously shown, retinopathy is the clinical symptom that was found to be most specifically linked to diabetes. The correlation between retinopathy and other clinical symptoms of interest are given in Tables 11 and 12. Those factors which showed the highest correlation coefficient, were selected for further analysis.

Since retinopathy seemed to be

Table 11 *Coefficients of correlation between retinopathy and different forms of diabetic complications and degree of familial occurrence of diabetes*

	Pulse freq	Syst blood pr	Diast blood pr	Puls feet	Calci ficat legs	Vibration perc	Ach jerk	Chol esterol	GTT
males	26	18	17	—08	16	05	10	20	—19
females	07	10	04	—22	19	—18	24	05	—25

	Serum creat	Proteinuria	Vision	Cataract	Hematuria	Age at manifest	Age	Duration	Famil occur of d m
males	22	40	—26	10	31	—29	—07	51	11
females	09	24	—47	29	10	—15	04	42	05

Table 12 *Coefficients of correlation between diabetic retinopathy and therapy level and fluctuation of urine sugar and blood sugar and tendency to acidosis with varying duration of the disease*

Duration (years)	Sex	Therapy	Urine sugar	Blood sugar	Fluctuat urine sugar	Fluctuat blood sugar	Acidosis tendency
1— 2	M	26	06	18	04	26	26
	F	08	04	15	—03	09	—01
5— 6	M	27	18	33	07	35	
	F	09	03	24	00	07	
10—11	M	18	21	31	02	25	
	F	10	10	—01	00	—16	
Total	M	33	19	28	05	29	14
	F	25	16	14	06	10	02
	M+F	29	17	20	06	20	10

Table 16 Age and degree of retinopathy in patients who had had diabetes for more than 12 years

Retinopathy (degree)	Age						Duration					
	Males		Females		Total		Males		Females		Total	
	n		n		n		n		n		n	
0	23	63.8	19	60.4	42	62.3	23	16.7	19	16.6	42	16.7
1	25	49.7	33	52.1	58	51.1	25	23.2	33	21.6	58	22.3
2	6	60.0	6	60.0	12	60.0	6	19.0	6	27.7	12	23.4
1+2	31	51.6	39	53.3	70	52.5	31	22.4	39	22.7	70	22.6
3	14	61.5	15	65.5	29	63.6	14	21.1	15	17.5	29	19.2
4	2	36.0	4	61.5	6	53.0	2	16.0	4	24.5	6	21.7
3+4	16	58.3	19	64.7	35	61.8	16	19.8	19	19.0	35	19.4

Table 17 Mean values, after 12 years duration of diabetes, for fatfree weight, bodyfat systolic blood pressure and pulse frequency in diabetics with different degrees of retinopathy compared with the mean values for series B

Retinopathy degree	Males				Females			
	0	1–2	3–4	mean series B	0	1–2	3–4	mean series B
Bodyweight								
60–79	73.7	72.5	79.3	7[illegible].9	59.4	73.9	72.9	67.9
40–59	66.5	70.9	74.5	71.3	68.3	60.5	59.6	61.9
20–39	—	66.5	71.0	66.8	61.4	60.1	63.5	61.8
total	68.9	70.0	74.9	72.8	63.0	64.8	65.3	63.9
Fatfree weight								
60–79	60.4	58.3	60.8	59.9	46.0	48.1	47.6	47.3
40–59	59.7	62.5	51.3	61.2	48.8	44.4	46.0	45.9
20–39	—	60.6	—	61.3	47.4	45.5	—	45.6
total	59.8	60.[illegible]	60.1	60.3	47.4	46.0	46.4	46.1
Bodyfat								
60–79	16.1	19.1	20.4	19.0	22.0	32.0	34.1	29.2
40–59	12.5	10.9	23.5	15.4	30.3	26.5	23.5	26.5
20–39	—	8.1	—	10.9	23.4	47.0	—	26.3
total	13.2	12.7	18.3	14.1	25.2	28.5	28.0	27.4
Systolic blood pressure								
60–79	168.8	185.4	186.3	168.8	181.8	182.5	177.9	177.9
40–59	147.5	152.9	157.5	152.0	183.5	161.7	195.0	166.9
20–39	—	137.5	—	144.1	—	151.3	—	141.7
total	153.5	158.6	162.6	155.0	170.0	165.2	170.7	162.2
Pulse frequency								
60–79	69.8	[illegible]9.6	80.5	70.3	82.8	82.6	88.1	82.7
40–59	[illegible]	75.4	86.0	7[illegible].3	83.5	89.5	80.5	86.5
20–39	—	80.1	—	73.6	—	83.5	—	89.4
total	74.1	78.4	81.7	75.4	85.2	85.2	86.0	86.2

Table 14 *Frequency (%) and distribution of different degrees of retinopathy in males and females*

All ages	n	Retinopathy degree 1	2	3	4	1—4
Males	215					
total frequency		18.1	4.2	7.0	0.5	29.8
rel. frequency		60.7	14.1	23.5	1.7	
Females	268					
total frequency		19.8	7.8	9.7	1.5	38.8
rel. frequency		51.0	20.1	25.0	3.9	
Males+Females	483					
total frequency		19.0	6.2	8.5	1.0	34.7
rel. frequency		54.8	17.9	24.5	2.9	
60—79 year						
Males	100					
total frequency		10.0	5.0	8.0	0	23.0
rel. frequency		43.5	21.7	34.8		
Females	162					
total frequency		15.4	8.6	13.0	1.9	38.9
rel. frequency		39.6	22.1	33.4	4.9	

Differences were noted with sex in the 60—79 year class where both the frequency and severity increased in the females (Table 14). The difference was of the same order as that found by LUNDBAEK (1953). Diabetes is also being discovered more often in elderly women than in men of corresponding age, which might suggest that factors of importance for the penetrance of diabetes are also of importance in the causation of complications. This is also supported to some extent by the frequency of diabetes among close relatives, which was found to be positively correlated with the degree of retinopathy (Table 15).

In the further analysis 2 groups were selected — those who had had the disease for less than 8 years and those who had had it for more than 12 years. In the former group which comprised only 31 patients with retinopathy, age matched patients who

Table 15 *Frequency (%) of known familial occurrence of diabetes in patients with different degrees of rentinopathy*

Retinopathy degree	n	Familial occurrence of d. m. degree 2—5	3—5
0	81	22.2	19.8
1—2	90	34.4	25.6
3—4	38	36.8	31.6

7 1le 18 D str l t o f lle t a lr c l l l c pl c l ns J l erso tl l ff re t legrees of r t i opatl y
l f r l o af le l ele

D g co l p hy		\ 0	1	2-4	w g	-2	3 4	Bo 0	1 2	3 1	fu qu y 0	1 2	3 4	\		3 4	0	1-2	1	l ot p 0	1	3 4	C 0	1-	3 1
l rat o > 12 ye rs																									
l s	+	7	3	0	3	10			13	C		14	1	1	1ɔ	11	10	12	8	ɔ	5	8	5	5	ɔ
	=	2	ɔ	1		4	ɔ	4	4	ɔ	3	3	0	0	3	0		3	4				14	21	7
	—	13	17	6	11	17	3	13	11	3	8	1?	1	10	13	2	7	6	ɔ	16	ʌ	6	4	1	4
C a s	+	8	21	13	8	14	11	C	26		7	18	13	10	21	11	3	13		3	2	5	3	15	8
	=	0	7	1	4	8	1	2	0	4	5	3	2	1	3	1	ɔ	9	2				1?	20	11
	—	11	1	ɔ	7	17	6	11	13	ɔ	C	16	3	8	1	7	7	1?	1	16	3	13	3	3	0
to al	+	1ɔ	30	2	17	21	10	11	13	15	6	??	?8	2	3f		13		13	8	7	13	8	20	13
	=	2	8		6	1	3	6	4	9	8	6		1	C	1		18	6				6	44	18
	—	21	3	11	18	31	1ɔ	21	?7	8	14	28	4	18	8		14	1?	6	3	60	?1		4	4
l at o < 8 ye s																									
t l	+	1	14	ɔ	10	11	4	15	1?	7	1?	10		16	13	4	11	1ɔ	ɔ	7	ɔ	1	0	ɔ	6
	=	4	1	—	6	8	6	3	4	0	4	6	4	?	4	1	0	0	1				16	10	1
	—	1	16	3	14	13	3	1?	13	0	13	11	1	13	14	1	1	3	0	23	2ɔ	8	6		0
t l	+	30	11	27	7	3ɔ	20	26	51	2?	28	42	30	38	49	29	21	40	18	5	1?	14	17	2ɔ	19
	=	C	0	?	1?	1ɔ	3	0	8	0	12	12	6	3	10	?		18	7				4	51	20
	—	3C	48	12	3?	48	18	36	40	8	7	3ɔ	7	31	4?	1	1ɔ	21	6		8	29	13	33	4

= 1 ot s ± 1 ɔ kg ± 10 k ± 1 % ± 1ɔ ± Hg
f ser B

had had the disease for less than 8 years without developing retinopathy served as controls

The average ages and duration of diabetes in patients with or without retinopathy is apparent from Table 16 which shows that the ages of the patients who had had the disease for more than 12 years varied betweeen the groups but was clearly lower in the group with micro aneurysms only The duration was largely uniformly increased in the group with different degrees of retinopathy compared to those without retinopathy

The mean values found for body weight, fatfree weight bodyfat, pulse frequency and systolic blood pressure in the various age groups in males and females, and with reference to the degree of retinopathy in the group who had had diabetes for more than 12 years, are given in Table 17, where a comparison is made with the mean values found for series B Mean values in the subgroups are given only if 5 o more observations had been made — in the calculation of the mean values of the means of the subgroups the values in these cases were supple mented with corresponding values from series B

Owing to the smallness of the series and differences between the age distri bution of the subgroups the values in Table 17 do not allow any definite evaluation Therefore values above below and equal to the mean values for corresponding ages and sex in series B were registered (Table 18)

The tables show that in the group with the severest retinopathy there was a distinct increase in bodyweight, which was in turn dependent on an increased amount of bodyfat The *tendency* was *most* marked in the higher age groups and contrasted with the general tendency *to reduction of* bodyfat with increasing duration of the *disease Conversely* there was a certain reduction of bodyfat among those who had had disease for more than 12 years without developing retinopathy It is clear from the tables that pulse frequency and systolic blood pressure were increased in the group with severe retinopathy which can be partly explained by the positive correlation between bodyfat pulse frequency and blood pressure which has been shown in both this series and in a normal population (NILSSON et al 1964)

As to calcifications of the arteries proteinuria and development of cata ract, there appeared to be a positive correlation between the occurrence of these conditions and the severity of retinopathy

It is still debatable to what extent careful regulation of the diabetes can retard or arrest the advance of diabetic complications While most investiga tions have shown that good regulation results in suppressed frequency of complications other workers have been unable to demonstrate such clear differences The materials described are as a rule difficult to judge from a statistical point of view — especially owing to differences in what is re garded as good and poor control Therefore in the present investigation no such classification was made but

only numerical expressions were used for the factors which might be of special interest in the description of the control of diabetes, namely treatment classified according to insulin requirements blood sugar level stability of adjustment expressed as degree of fluctuation of the blood sugar level, and tendency to acidosis The basis of this classification is described in 'Methods Table 19 is based on the same principle as Table 18 The table shows that especially in the group that could be judged better i e that had had the disease longer the insulin requirement was larger and the blood sugar level fluctuated markedly in patients with different degrees of retinopathy, while the blood sugar level during the entire development of the disease appeared to be definitely elevated in those in whom more severe changes were found in the ocular fundi Table 20 shows that the frequency of both mild and severe signs of acidosis did not vary with the severity of retinopathy but it was higher in those with than in those without retinopathy

Table 20 *Frequency (%) of acidosis of varying severity among diabetics with different degrees of retinopathy*

Degree of retinopathy	n	Degree of acidosis	
		2—4	3—4
0	51	29 4	5 9
1—2	66	51 5	10 0
3—4	35	48 6	11 4

Table 19 *Distribution of therapy and level and fluctuation of blood sugar with different degrees of retinopathy and duration of diabetes*

Degree of retinopathy		Therapy after 1 2 years			Bloodsugar after 1 2 years			Therapy after 5 6 years			Bloodsugar after 5 6 years			Therapy after 10 11 years			Bloodsugar after 10 11 years			Therapy total			Bloodsugar total			Fluctuation bloodsugar total		
		0	1 2	3 4	0	1-2	3 4	0	1 2	3 4	0	1-2	3 4	0	1-2	3 4	0	1 2	3 4	0	1 2	3 4	0	1-2	3 4	0	1 2	3 4
Duration																												
< 12 yrs	+	11	13	11	4	8	9	9	16	10	8	6	9	9	16	10	5	9	11	9	17	10	7	9	12	6	18	12
males	=	0	4	3	3	1	0	3	1	3	2	1	2	4	3	3	3	1	0	7	[illegible]	5	3	5	1	3	5	2
	—	10	5	2	8	8	4	10	6	1	9	14	2	9	7	1	9	14	3	7	8	1	9	14	3	12	5	2
	+	7	12	8	6	11	9	6	8	7	3	9	10	4	12	5	11	14	11	5	27	11	11	28	12	9	28	9
females	=	1	2	1	0	2	0	1	1	4	3	1	1	4	7	6	1	6	0	7	3	3	3	1	2	2	2	1
	—	5	6	3	8	7	3	3	7	1	4	4	1	10	9	8	6	8	7	6	9	5	5	11	4	8	17	9
	+	18	25	19	10	19	18	15	21	17	11	15	19	13	28	15	16	23	22	14	44	21	18	37	21	15	46	21
total	=	1	6	4	3	3	0	4	2	7	5	2	3	8	10	9	4	7	0	14	8	8	6	6	3	5	7	3
	—	15	11	5	16	15	7	13	13	2	13	18	3	19	16	9	15	22	10	13	17	6	14	25	7	20	22	11
Duration																												
< 8 yrs	+	9	3	3	14	11	4													10	5	4	10	10	3	14	10	3
total	=	17	14	5	4	1	0													14	3	3	13	1	0	2	1	1
	—	5	3	0	13	8	4													7	14	1	12	11	5	15	11	4
	+	27	28	22	24	30	22													24	49	25	34	47	27	23	56	24
total	=	18	20	9	7	4	0													28	11	11	29	7	3	7	8	4
	—	20	14	5	29	21	11													20	31	7	26	36	12	35	33	15

Table 22 *Frequencies (%) of varying degree of the sense of vibration of the malleoli in different ages in diabetes of short (A) and long (B) duration compared with normal values (N)*

Series	Intact			Partly intact			Absent		
	N	A	B	N	A	B	N	A	B
Males									
60—79	71.4	34.3	40.0	17.1	37.1	30.0	11.4	28.6	30.0
40—59	95.2	91.4	88.0	4.8	2.9	7.3	0	5.8	4.9
20—39	100.0	100.0	100.0	0	0	0	0	0	0
mean	88.9	75.2	71.6	7.3	13.3	12.4	3.8	11.5	11.6
Females									
60—79	92.6	71.0	40.0	7.4	21.1	25.0	0	7.9	35.0
40—59	94.1	76.6	76.7	0	20.0	23.3	5.9	0	5.3
20—39	100.0	100.0	100.0	0	0	0	0	0	0
mean	95.6	82.5	72.2	2.5	13.7	17.9	2.0	3.7	11.7
M+F mean	92.3	78.9	74.1	4.9	13.5	15.2	2.9	7.6	11.7

clearly with the duration of the disease, but was increased also in recently discovered diabetes

Vascular status

The vascular status was judged by palpation of the pulsations in the arteries of the foot and x ray examination of the legs for calcifications of the arteries (Tables 23 and 24) The most striking correlation was found between calcifications and duration — An increased tendency to calcifications has been reported in diabetes of long duration in patients in whom the disease had made its appearance in childhood (WHITE 1960)

Table 23 *Frequencies (%) of different degrees of pulsations of feet arteries in different ages in diabetes of short (A) and long (B) duration compared with normal values (N)*

Series	Palpable			Partly palpable			Not palpable		
	N	A	B	N	A	B	N	A	B
Males									
60—79	60.0	40.0	45.0	37.1	28.6	22.5	2.9	31.4	32.5
40—59	95.2	80.1	66.7	4.8	8.3	26.2	0	11.1	7.1
20—39	96.8	90.0	100.0	3.2	10.0	0	0	0	0
mean	84.0	70.0	70.6	15.0	15.6	16.2	1.0	14.2	13.2
Females									
60— 9	60.7	52.6	33.3	32.1	31.6	51.3	7.1	15.8	15.4
40—59	80.6	76.7	53.3	13.9	23.3	46.7	5.6	0	0
20—39	88.2	81.8	73.7	11.8	18.2	4.1	0	0	5.3
mean	6.5	0.4	53.4	19.3	24.4	34.0	4.2	5.3	6.9
M+F mean	80.3	70.2	62.0	17.2	20.0	25.1	2.6	9.8	10.1

VIII. Various clinical observations in diabetics

As recently stressed by DANOWSKI et al. (1966), it is difficult to compare the frequencies of clinical symptoms found in different diabetic series. This is due mainly to differences in the subjective interpretation of the findings — e.g. evaluation of reflex, determination of perception of vibration, registration of arterial pulsations and to some extent also interpretation of the roentgenograms, urinary sediment, cataract etc. Such difficulties also apply to the present investigation. Therefore of greater interest than comparisons with other materials is a comparison between a group of normal controls and diabetics who have had the disease for a short respectively long period.

Neurological status

The neurological status, here elucidating only the state of the peripheral nerves of the lower limbs, was judged by estimation of the achilles reflex and the perception of vibration over the malleoli. The results are given in Tables 21 and 22. The values agree largely with those reported by LUNDBAEK (1953), STEINESS (1957) and MAYNE (1965). The frequency of pathological neurological signs varied

Table 21. *Frequencies (%) of presence and absence of achilles jerk in diabetes of short (A) and long (B) duration compared with normal values (N)*

	Present			Partly absent			Absent		
Series	N	A	B	N	A	B	N	A	B
Males									
60—79	75.0	45.9	32.5	3.6	27.0	40.0	21.4	27.0	27.5
40—59	92.9	75.0	52.4	2.4	13.9	33.3	4.8	11.1	14.3
20—39	0	90.0	76.2		10.0	14.3	0	0	9.5
mean	89.3	70.3	53.7	2.0	17.0	29.2	8.7	12.7	17.1
Females									
60—79	71.4	57.5	12.8	3.6	27.5	53.8	25.0	15.0	33.3
40—59	97.2	53.3	53.3	2.8	36.7	40.0	0	10.0	6.7
20—39	0	84.6	78.9	0	15.4	21.1	0	0	0
mean	89.5	65.1	48.3	2.1	26.5	38.3	8.3	8.3	13.3
M+F mean	89.4	67.7	51.0	2.1	21.8	33.8	8.5	10.5	15.2

Table 26 *Frequencies (%) of ocular tonus of various degree in different ages in diabetes of short (A) and long (B) duration compared with normal values (N)*

Degree	< 15 mm Hg			15—19 mm Hg			> 19 mm Hg		
Series	N	A	B	N	A	B	N	A	B
Males									
60—79	37.2	22.2	16.8	45.7	51.9	50.0	17.1	25.9	33.3
40—59	38.1	20.0	6.9	47.6	46.7	65.5	14.3	33.3	27.6
20—39	34.5	26.7	7.7	58.6	40.0	76.9	6.9	33.3	15.4
mean	36.6	23.0	10.5	50.6	46.2	64.1	12.8	30.8	25.4
Females									
60—79	17.8	23.3	8.4	67.9	46.7	45.8	14.3	30.0	45.8
40—59	17.2	12.5	8.0	77.1	25.0	40.0	5.7	62.5	52.0
20—39	32.4	0	7.1	52.9	63.6	50.0	14.7	36.4	42.9
mean	22.4	11.9	7.8	66.0	45.1	45.3	11.6	43.0	46.9
M+F mean	29.5	17.5	9.2	58.3	45.7	54.7	12.2	36.9	36.1

Table 27 *Frequencies (%) of arcus lipoides corneæ of various degree in different ages in diabetes of short (A) and long (B) duration compared with normal values (N)*

Degree	0			slight or moderat			Pronounced		
Series	N	A	B	N	A	B	N	A	B
Males									
60—79	42.8	22.2	20.0	48.6	55.6	43.3	8.6	22.2	36.7
40—59	64.2	63.3	48.8	31.0	36.7	37.9	4.8	0	13.3
20—39	93.1	93.3	6.9	6.9	6.7	23.1	0	0	0
mean	66.7	59.6	48.5	28.8	33.0	34.8	4.5	7.4	16.7
Females									
60—79	42.9	25.8	45.8	57.1	48.4	41.7	0	25.8	12.5
40—59	70.6	75.0	77.0	29.4	25.0	19.2	0	0	3.8
20—39	91.2	80.9	100.0	8.8	19.1	0	0	0	0
mean	68.2	63.9	74.3	31.8	27.5	20.3	0	8.6	5.4
M+F mean	67.5	61.7	61.4	30.3	30.3	27.6	2.3	8.0	11.0

was noted in 3.2 % of the controls in the 60—79 year age class compared with 4.5 % in diabetics

Arcus lipoides corneæ (see Table 27) in males tended to increase in frequency with the duration of the disease But since the observation could not be confirmed in the women its significance is questionable

Table 24 *Frequencies (%) of calcifications of various degrees (see Methods) of lower limbs in different ages in diabetes of short (A) and long (B) duration compared with normal values (N)*

Degree	0			1—2			3—4			5—6		
Series	N	A	B	N	A	B	N	A	B	N	A	B
Males												
60—79	22 2	12 5	2 9	36 1	28 1	14 3	22 2	15 6	25 7	19 4	43 8	51 1
40—59	64 3	41 4	22 2	16 7	41 4	38 9	9 5	17 2	27 5	9 5	0	11 1
20—39	96 8	93 3	68 4	3 2	6 7	15 8	0	0	10 5	0	0	5 3
mean	61 1	49 1	31 2	18 7	25 4	23 0	10 6	10 9	21 2	9 6	14 6	24 5
Females												
60—79	57 1	30 6	9 7	35 7	47 2	32 3	7 1	13 9	35 5	0	8 3	22 6
40—59	94 6	67 9	51 9	0	32 1	25 9	5 4	0	11 1	0	0	11 1
20—39	100 0	100 0	76 9	0	0	7 7	0	0	15 4	0	0	0
mean	83 9	66 2	46 2	11 9	26 4	22 0	4 2	4 6	20 7	0	2 8	11 2
M+F mean	72 5	57 7	38 7	15 3	25 9	22 5	7 4	7 8	21 0	4 8	8 7	17 9

Table 25 *Frequencies (%) of cataract of various degrees in different ages in diabetes of short (A) and long (B) duration compared with normal values (N)*

Degree	0			Peripheral not affecting vision			Moderate or dense		
Series	N	A	B	N	A	B	N	A	B
Males									
60—79	42 9	40 8	25 8	37 1	33 3	58 1	20 0	25 9	16 1
40—59	90 5	90 0	73 3	9 5	6 7	20 0	0	3.3	6 7
20—39	100 0	100 0	84 6	0	0	7 7	0	0	7 7
mean	77 8	77 0	61 2	15 5	13 3	28 6	6 7	9 7	10 2
Females									
60—79	39 3	47 1	16 0	50 0	41 1	52 0	10 7	11 8	32 0
40—59	88 6	82 1	48 2	11 4	14 3	37 0	0	3 6	14 8
20—39	100 0	91 7	77 7	0	8 3	5 6	0	0	16 7
mean	78 4	72 0	47 3	18 0	21 2	31 5	3 6	6 8	21 2
M+F mean	78 1	74 5	54 2	16 8	17 3	30 1	5 2	8 3	15 7

Ocular status

Diabetic retinopathy has been discussed in Chapter VII As shown by KORNERUP (1955), the frequency of *cataract* increases markedly with the duration of diabetes (Table 25) In the lower age classes the cataract has some specific characteristics, while in the higher age classes it cannot be distinguished from ordinary senile cataract Since the frequency of cataract is hardly increased in newly discovered cases of diabetes it is probably dependent on the metabolic disturbances due to diabetes

Tonus appears to be somewhat increased in diabetics — but the increase is not correlated with the duration of the disease (See Table 26) An ocular pressure of more than 25 mmHg

Renal status

The state of the kidneys was judged according to presence or absence of proteinuria, erythrocytes and leukocytes in the urinary sediment, and serum creatinine. In clinical investigations of this type it is difficult to reveal incipient renal injury of specific diabetic type and of pyelonephritis (THOMSEN 1956, HALVERSTADT et al 1966).

The investigations demonstrated in Table 29 shows a distinct quantitative and qualitative correlation between *proteinuria* and the duration of diabetes. It is clear from the correlation coefficient in Table 9, too, that covariation was closer in the males. The situation was roughly the same for hæmaturia (Table 30) but the frequencies were too low to assess the significance of the finding.

The *serum creatinine* (Table 31) did not vary with the duration of the diabetes and values within the normal range are presumably of little value in the diagnosis of incipient renal disease.

Table 30 *Frequencies (%) of hematuria of various degree in different ages in diabetes of short (A) and long (B) duration compared with normal values (N)*

	0—1 cells/field			2—10 cells/field			> 10 cells/field		
Series	N	A	B	N	A	B	N	A	B
Males									
60—79	97.5	90.0	94.6	2.5	10.0	2.9	0	0	2.9
40—59	100.0	97.0	100.0	0	3.0	0	0	0	0
20—39	97.5	100.0	85.0	2.5	0	5.0	0	0	10.0
mean	98.3	95.7	93.1	1.7	4.3	2.6	0	0	4.3
Females									
60—79	97.5	97.5	92.5	2.5	2.5	7.5	0	0	0
40—59	100.0	93.3	90.0	0	3.3	10.0	0	3.3	0
20—39	95.2	100.0	88.9	4.8	0	11.1	0	0	0
mean	97.6	96.0	90.5	2.4	2.9	9.5	0	1.1	0
M+F mean	97.9	95.8	91.3	2.1	3.6	6.1	0	0.6	2.1

Table 31 *Serum creatinine (mg/100 ml) in diabetes of short (A) and long (B) duration*

Series / age	Males A mean	Males B mean	Males A+B SD	Females A mean	Females B mean	Females A+B SD	Males+Females A mean	Males+Females B mean	Males+Females A+B SD
60—79	0.99	1.01	0.25	0.84	0.88	0.26	0.92	0.95	0.26
40—59	0.86	0.79	0.42	0.74	0.71	0.34	0.85	0.75	0.40
20—39	0.89	0.93	0.34	0.63	0.66	0.31	0.74	0.80	0.33
mean	0.91	0.91		0.75	0.75		0.81	0.83	

Table 28 *Systolic and diastolic blood pressure in diabetes of short (A) and long (B) duration compared with normal values (N)*

Systolic pressure	Males					Females					Females Males+		
series / age	N mean	SD	A mean	B mean	A+B SD	N mean	SD	A mean	B mean	A+B SD	N mean	A mean	B mean
60—79	164 4	22 5	166 8	168 8	27 5	173 4	21 0	179 3	177 9	25 9	168 9	173 1	173 4
40—59	142 3	14 9	155 9	152 0	20 2	143 8	17 9	163 0	166 8	24 8	143 1	159 5	159 4
20—39	127 7	15 4	132 8	144 1	20 8	126 5	14 8	143 2	141 7	18 0	127 1	138 0	142 9
mean	144 8		151 8	155 0		147 9		161 8	162 1		146 4	156 9	158 6
Diastolic pressure													
60—79	90 3	11 8	100 0	97 5	12 5	93 2	9 5	96 1	93 4	12 4	91 8	98 1	95 5
40—59	86 1	8 5	100 8	95 8	12 2	83 9	7 6	94 0	93 5	11 4	85 0	97 2	94 7
20—39	76 6	7 8	89 0	92 5	11 9	79 9	8 3	84 6	85 7	8 7	78.3	86 8	89 1
mean	84 3		96 6	95 3		85 7		91 6	90 9		85 0	94 0	93 1

Blood pressure

The blood pressure, and particularly the diastolic pressure, tended to be higher among diabetics than among the controls (Table 28) On the other hand, it did not appear to be appreciably correlated with duration of diabetes A tendency to a covariation was noted between the development of retinopathy and increased blood pressure (Tables 11 and 17) The diabetics did not differ from the controls in respect of the frequency of fundus hypertonicus No correlation was found between retinopathy due to hypertension and that due to diabetes

Table 29 *Frequencies (%) of proteinuria of various degree in different ages in diabetes of short (A) and long (B) duration compared with normal values (N)*

	0			0 1—0 3 g/1000 ml			≥ 0 4 g/1000 ml		
Series	N	A	B	N	A	B	N	A	B
Males									
60—79	91 7	74 2	55 3	8 3	25 8	36 8	0	0	7 9
40—59	97 6	97 0	89 8	2 4	3 0	5 3	0	0	4 9
20—39	100 0	100 0	63 7	0	0	22 7	0	0	13 6
mean	96 4	90 4	69 6	3 6	9 6	21 6	0	0	8 8
Females									
60—79	96 4	90 0	75 0	3 6	10 0	17 5	0	0	7 5
40—59	97 6	86 7	86 7	0	10 0	10 0	2 4	3 3	3 3
20—39	100 0	92 9	100 0	0	7 1	0	0	0	0
mean	98 0	89 9	87 2	1 2	9 0	9 2	0 8	1 1	3 6
M+F mean	97 2	90 1	83 5	2 4	9 3	10 3	0 4	0 6	6 2

IX Glutamic-Pyruvic-Transaminase (GPT)

The values found in the investigation are given in Table 34 The GPT values noted in the controls were for some unknown reason somewhat low and are therefore less suitable for direct comparison with those noted in the diabetics In the beginning of the investigation the GPT was taken as a measure of liver damage and it was expected to be increased with the duration of the disease because liver damage has been said to develop in long standing diabetes (ENGLESON 1954)

In contrast to what was expected however a negative correlation was found between the GPT and the duration of diabetes (Table 9) Of the newly discovered cases in series A 16 of 177 had a GPT of more than 40 units while in series B only one had such a high value The increases were found particularly in the 40—59 year group where they were seen in 8 men and 4 women *On supplementary analysis* to find an explanation for this negative correlation the following findings were made

1 Treatment with oral anti diabetics has been reported as being able to produce liver injury In the material the GPT in those who received tablets was not higher than in those who were treated with insulin or by dietary measures only Of the 16 with GPT of more than 40 units there were thus 10 who received tablets, a number not differing significantly from what was expected
2 Liver injury in the form of cirrhosis in 3 cases and hepatitis in one was noted among those with a GPT of more than 40 units The

Table 34 *Glutamic pyruvic transaminase (units/ml) in different ages in series A and B compared with normal values*

Series / age	Males				Females				Males+Females			
	N	A	B	SD (A+B)	N	A	B	SD (A+B)	N	A	B	SD (A+B)
60—79	6.6	17.7	13.4	9.8	4.8	16.6	9.0	10.4	5.7	17.2	11.2	10.2
40—59	9.0	28.3	14.0	16.8	3.7	25.1	15.4	13.4	6.4	26.7	14.7	15.4
20—39	10.5	21.8	14.4	13.6	9.0	10.7	10.9	12.2	9.8	16.6	12.7	12.9
mean	8.7	22.6	13.9	13.6	4.6	19.0	10.2	11.5	6.7	20.8	12.1	13.3

Table 32 *Frequencies (%) of pyuria of various degree in different ages in diabetes of short (A) and long (B) duration compared with normal values (N)*

Series	0—1 leucocytes/field			2—10 leucocytes/field			> 10 leucocytes/field		
	N	A	B	N	A	B	N	A	B
Males									
60—79	67.8	57.1	58.3	29.4	42.9	30.6	2.8	0	11.1
40—59	78.1	57.7	75.8	21.9	42.3	24.2	0	0	0
20—39	71.0	79.0	75.0	29.0	10.5	15.0	0	10.5	10.0
mean	72.3	64.6	69.7	26.7	31.9	23.3	0.9	3.5	7.0
Females									
60—79	67.8	42.5	51.3	28.6	40.0	30.8	3.6	17.5	17.9
40—59	72.3	55.2	55.0	22.2	37.9	20.0	5.5	6.9	25.0
20—39	64.7	50.0	64.7	29.4	42.9	23.5	5.9	7.1	11.8
mean	68.3	49.2	57.0	26.7	40.3	24.8	5.0	10.5	18.2
M+F mean	70.8	56.9	63.2	26.7	36.1	24.0	3.0	7.0	12.6

The frequency of *pyuria* (Table 32) *appears to be* increased among diabetics but does not vary with the duration of diabetes, which *suggest that this symptoms should not be referred to* diabetic organic injury

Cholesterol

The serum cholesterol was not determined in our controls The values determined by LINDHOLM (1956) were therefore used as standards In his investigation the cholesterol was measured *according to a modification* of the method described by SCHOENHEIMER & SPERRY (*1934*) This material was collected from largely the same population but *according to* different sampling criteria, which may be expected to result in somewhat lower mean values The serum cholesterol tended to increase with the duration of diabetes (Table 33) Particularly the males showed a positive correlation between *the serum cholesterol and the* frequency of diabetic retinopathy (Table 11) — correlations of the same order have also been observed between the cholesterol and proteinuria respectively hematuria

Table 33 *Serum cholesterol (mg/100 ml) in different ages in series A and B compared with normal values (LINDHOLM 1956)*

Series / age	Males				Females				Males+Females			
	N	A	B	SD (A+B)	N	A	B	SD (A+B)	N	A	B	SD (A+B)
60—79	180	191	194	37	218	203	210	59	199	197	202	46
40—59	180	207	204	46	191	206	227	53	186	207	216	49
20—39	167	181	195	57	169	175	180	21	168	178	188	45
mean	177	193	198	45	193	195	206	51	184	194	202	48

X Discussion

As pointed out by JOSLIN (1959) Sweden lends itself well to population studies capable of yielding valuable information on diabetes mellitus Thanks to the national health scheme and special health surveys it may be assumed that practically all cases of diabetes are discovered In the rural districts the regulation of diabetics is carried out mainly at regional hospitals where the patients are examined at least a few times a year Owing to this organisation we have been able to examine a complete population of diabetics and have thus escaped the sampling problems that make it difficult to evaluate most other studies in this field In the evaluation of the diabetics we also had at our disposal — in contrast to other workers — an adequate control series with which valid statistical comparisons could be made

The results of the investigation not only confirmed several well known clinical observations concerning anthropometric factors complications and treatment but also elucidated new aspects of the picture of diabetes especially through the use of a simple anthropometric method for estimating bodyfat and fatfree weight

As previously shown (STEINBERG 1959 1961 NILSSON 1962) it is probable that the main cause of diabetes is a recessively inherited gene of hitherto unknown nature The genetic statistical analysis is complicated by the fact that not all carriers of the gene for diabetes develop the disease and that even penetrance factors are to some extent inherited The importance of different penetrance factors in different social environments and during different periods of life is reflected among other things by the change of the ratio between the sexes observed during the last decade (MARTINS et al 1965) and confirmed in the present material This change seems to be due at least partly to a relative decrease in the morbidity of middle aged women

Among persons developing the disease there is an increased representation of mesomorphy which implies an increase of the fatfree weight and especially of the factor which LINDEGÅRD (1953) calls sturdiness As shown also by other investigators (SELTZER & MAYER 1964 ALRELL et al 1966) this sturdiness factor varies to a certain extent with the tendency to obesity this correlation being strongest in middle age

frequency of known liver injury — usually cirrhosis — in the entire material was 3 %

3 Heart injury in the form of previous myocardial infarction was noted in 2 cases, besides which one of those who had a GPT of more than 40 units had a negative T in the lateral chest leads

4 In the controls (NILSSON et al 1964) the amount of bodyfat and the GPT tended to vary with one another Such a positive correlation was found among diabetics and the coefficient was highest in the 40—59 year group, where the coefficient for the male diabetics was 0 32 and for the females 0 24 The amount of bodyfat in patients with GPT of more than 40 units was, on the average, increased by 3 %

5 It is also possible that an increased mortality among persons with increased GPT contributed to the differences observed

XI Summary

Observations made in an investigation of a representative diabetic population and compared with those made in an adequate control series appeared to allow the following conclusions

1 At discovery of the disease diabetics are overweight owing to an increase of both fatfree weight and bodyfat
2 The amount of bodyfat decreases with the duration of the disease with the result that bodyweight becomes subnormal
3 Of the clinical symptoms investigated retinopathy is most closely correlated with the duration of diabetes The next highest correlation coefficient is that of calcification of the arteries of the lower limbs
4 The frequency of retinopathy reaches its maximum after about 16 years and then remains fairly constant
5 Diabetics with retinopathy are characterised by an increased amount of bodyfat, increased pulse frequency increased blood pressure which factors are also intercorrelated with one another In such patients the insulin requirement is increased the blood sugar level is elevated and there is an increased tendency to acidosis
6 The severity of retinopathy varies with the degree of obesity and the extent of the elevation of the blood sugar level
7 Vascular and neurological signs in the lower limbs are closely correlated with one another
8 The serum glutamic pyruvic transaminase decreases in both sexes and in all ages with increasing duration of the diabetes

The reported frequencies of symptoms of diabetic complications vary with the possibility of their detection The complication most readily discovered is retinopathy, with the result that various factors of importance for the development of complications can be analysed best by examination for any correlation with changes in the ocular fundi Such an analysis showed that diabetics who had had the disease for a long time without complications were those with decreased amount of bodyfat, which has been pointed out by ENGEL (1950) On the other hand, those who had the most advanced retinopathy showed a remarkable increase of fat and, on the average, an elevated blood sugar level These findings underline the value of dietary restrictions in the prevention of complications and should be seen in the light of the decreased frequency of diabetes and complications recorded during periods when food was rationed, such as during the last two wars With the present dietary regimen however the frequency of blindness and severe impairment of vision is low, and in the population studied it was about 2 % Impairment of vision in diabetics is due to an equally large extent to cataract as to changes in the ocular fundi

Renal disease was relatively rarely noted in the present investigation This does not necessarily mean that diabetic nephropathy was uncommon but only that the clinical investigations were inadequate and that renal disease rarely has any obvious effect on the general physical condition

Other clinical symptoms noted in the present investigation and referable to the vascular system peripheral nerves and eyes could hardly be distinguished from those occurring with age also in non diabetics The changes are probably owing to specific diabetic complications accelerating the process of ageing The clinical symptoms found to vary closest with the duration of diabetes and the presence or absence of retinopathy were calcifications of the arteries of the lower limbs and ocular cataract

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— A Population Study of Men Born in 1913

BY

GÖSTA TIBBLIN

GÖTEBORG 1967

ACTA MEDICA SCANDINAVICA
SUPPLEMENTUM 470

FROM THE DEPARTMENT OF MEDICINE I (PROFESSOR L. WERKÖ) SAHLGRENSKA HOSPITAL, UNIVERSITY OF GÖTEBORG, GÖTEBORG, SWEDEN

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ELANDERS BOKTRYCKERI AKTIEBOLAG

CONTENTS

Studies of hospital series

A review of all the important literature on high blood pressure can be found in the monographs of Pickering (129) and Smirk (148) The literature on the physiology of high blood pressure has recently been reviewed by Page and McCubbin (122) Therefore no attempt will be made to review in detail the literature regarding studies of hospital series

Population studies

In studies of populations including both the diseased and healthy it is possible to complete the clinical picture and natural history of a disease The phrase is from Morris (110a) and in the same book he has given some reasons for using the epidemiological method The clinician is limited in his picture of the chronic disease Numbers can be large and yet the clinician s experience may be incomplete and the patients he deals with unrepresentative of all types and degree i e the picture may be biased The reasons for this are manifold in personal specialism and reputation in geographic economic and administrative situations Such limitations apply also to the work of a hospital or any other medical faculty Selective processes affecting admission to hospital may be explicit and obvious in terms of severity for example They can also be subtle and complicated and to make things worse may be working unsuspected and in manner undefined All this is to refer only to patients who present to medical attention But even advanced disease may be symptomless and even those suffering severe disability may not seek help, at the other extreme much mild illness is ignored The epidemiologist concerned with the total of ascertainable *cases in a specified population and not* merely with patients who present in particular hospitals clinics or practices, can help to provide a fuller picture than is obtainable in any or all of these This fuller picture may also prove to be a different one Morris illustrated how hospital experience gives an incomplete picture of the disease He was puzzled at the rarity of ruptured ventricle of the heart in the pathological archives of the London Hospital despite the numerous examples of other manifestations of ischaemic heart disease The answer eventually was supplied to him when he found that the coroner s pathologist saw two cases a week Less than 15 per cent of the total number of ruptured ventricles *of the heart were recognized in hospitals and when the hospital series* were compared with the total picture of ruptured ventricle the hospital picture was wrong on size sex and age

In the past 20 years there has been a notable increase of the interest in chronic diseases such as coronary heart disease and hypertensive disease Only relatively recently has the possibility of acquiring knowledge by applying epidemiological *methods to such* studies been used

Within the last 15 years representative population studies have been undertaken in widely scattered parts of the world The present review will deal with some of the studies of representative white populations in which blood pressure has been a main point of interest Extensive reviews of the studies of the epidemiology of high blood pressure of unknown cause have recently been written by Geiger and Scotch (60), Paul and Ostfeld (126), and Epstein (50)

U S A

The first published study of blood pressure which attempted a representative sampling of the entire population was based upon a 2% sample of the population in Muscogee County, Georgia (28) Of the selected sample of 1912 subjects, observations were obtained on 61% The examined population was composed of persons 8 years of age and older There was a general tendency for the mean blood pressure to increase with age, more marked for systolic than for diastolic blood pressure

The Framingham Study was established between the years 1948 and 1950 with the purpose of following a sample of a general population group for a period of some 20 years to study the characteristics associated with the development of high blood pressure and coronary heart disease (34) (77) A random sample consisting of twothirds of those persons in the town of Framingham, Massachusetts, was selected Of the 6510 subjects a total of 4469 (68 7%) agreed to participate In both sexes systolic and diastolic blood pressure rose with age (76) The rise with age was significantly greater in females than in males In both sexes, and at all ages, the higher the blood pressure, the greater was the prevalence of cardiac enlargement There was no evidence to indicate a sharp line of demarcation between normotensive individuals and those with hypertension

In Tecumseh, Michigan, Epstein and co workers during 1959–1960 made a study of the total population (49) The town has a population of approximately 9500, and it is considered representative in its social and economic structure of a small, midwestern community The response rate was over 90% among the younger age groups and decreased to just over 70% for persons past 70 years of age The blood pressure distributions were unimodal, generally symmetrical, but progressively more skewed to the right with age The findings were considered compatible with the concept of the interplay of multifactorial genetic and environmental factors determining blood pressure (52)

During the years 1960 to 1962, a survey of cardiovascular conditions was conducted among the population of Evans County Georgia (98) The study population identified by census, consisted of all persons aged 40 to 74 and a 50% random sample of those aged 15 to 39 Of the 3377 subjects in the study population 92% were examined Two indexes of hypertensive disease were used left ventricular hypertrophy (ECG) and cardiac enlargement determined from a reading of a posterior—anterior chest film The relationship between casual blood pressure determination and these signs was studied and the conclusion was reached that a single casual blood pressure determination was a valid index of hypertensive disease

in this population group (99) Over the age range studied mean systolic pressure continued to rise *with age whereas mean* diastolic pressure rose until ages 45 to 54 For the older ages no appreciable further rise occurred This suggests that the processes determining systolic and diastolic blood pressure differ The continuing increase in systolic pressure with age probably represents according to the authors the decreasing resilience of the aorta and its branches

Europe

In 1954 Miall and Oldham carried out a survey of arterial blood pressure in an urban and a rural population in South Wales (107) Blood pressure was measured in 623 subjects representing over 95% of the population sample Measurements were made under approximately standard circumstances by one observer The results showed that those engaged in light occupations had higher blood pressures than those doing heavy manual work Between the ages of 15 and 44 years married women without children had higher pressures than those with one child, who in turn had higher pressures than those with two or more children Single men and women had higher systolic pressure than those who were married The authors concluded that blood pressure is a physical characteristic analogous to obesity for example Both are continuously variable with no natural boundary separating normal from abnormal both when increased are associated with increased risks and both are probably influenced in a similar manner by environmental and genetic factors

Boe Humerfelt and Wederwang carried out a mass radiography and blood pressure investigation in Bergen Norway during the years 1950–1951 (20) Blood pressure readings were obtained from a total of 67 976 persons (27 718 males and 40,258 females) This was 70% and 82% respectively of all adults in the city Humerfelt reexamined a subsample of these subjects (70) This time 83% of the males and 79% of the females attended In analysing the data he separated as completely as possible the effects of age height and weight on blood pressure Age was by far the most important factor Height alone had a negligible effect For constant height and age, blood pressure increased slightly with increasing weight and the relation was approximately linear within the observed range The systolic blood pressure increased only about 3 mm for each 10 kg increase in weight, and the diastolic blood pressure only 2 mm The author emphasized that this was in marked contrast to the common belief that there was a strong positive correlation between blood pressure and excess weight

In eastern and western rural areas of Finland Karvonen and co-workers (1956) studied blood pressure by means of epidemiological field surveys (82) A number of small communities were selected and the local public health nurses invited men aged 20–59 to participate (civil servants temporary residents and those known to be ill were excluded) Very few refused to co-operate In the analysis of the results those with dietary peculiarities clinical evidence of cardiac or thyroid disease, subjective symptoms suggesting cardiac disease or physical disabilities were excluded Systolic and diastolic blood pres-

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sure were found to be slightly higher in the east than in the west of Finland A second field study was carried out 1959 in the same regions as the first study More than 800 men were examined in each region and a coverage of 97–98 per cent of all men between 40 and 59 years of age was attained This study confirmed the results of 1956, both systolic and diastolic pressure were significantly higher in the east than in the west The distribution of systolic blood pressure was unimodal in each region For diastolic blood pressure, however, the distribution for the east was somewhat irregular, although it could hardly be called bimodal

In Knkala, a rural community in the southwest of Finland, Tuomi investigated blood pressure in 41- to 60 year old subjects in 1960–1961 (161) The majority were manual labourers Of the expected number, 96 5% were examined, using methods suggested by the World Health Organization The mean blood pressure in men, 136 5/83 7 mm Hg, corresponds with the figures previously obtained in rural parts of Finland The mean blood pressure in men smoking more than half a pack of cigarettes a day was significantly lower than in the non smokers The ECG criterion for left ventricular hypertrophy, R_{V_5} 26 mm or over, was observed significantly more frequently in the hypertensive than in the other blood pressure groups In the group of over weight subjects the mean blood pressure was clearly higher than that of the whole group, according to the author, one of the causes of this was the larger circumference of the upper arm

Keys and collaborators (85) have recently reported data on variables presumed to be relevant to the epidemiology of coronary heart disease One of these variables was blood pressure The data was from 17 samples of men aged 40 through 59 in the U S A, Japan, Yugoslavia, Finland, Italy, the Netherlands and Greece Thirteen of these samples represent all men of given age in different rural areas villages and a small town (Zutphen, the Netherlands) The report concerned the findings in the cross section surveys

Summary

The studies reviewed are all from rural communities or small towns When using casual blood pressure measurements it has been shown that the blood pressure is distributed continuously in the population and that it is artificial to fix a precise borderline between those with high blood pressure and those without The average systolic and diastolic pressure for different groups increases with age in both sexes The systolic blood pressure increases more than the diastolic blood pressure after age 50 In all populations the blood pressure is lower in young women than in young men and higher in old women than in old men The findings from all these population studies undertaken in scattered parts of the world are built only upon measurements of blood pressure in the general population Operational difficulties for investigation of cases with high blood pressure of known cause have been so great as to make them totally inapplicable in epidemiological field situations Because of the difficulty in excluding them it follows that the epidemiology of high blood pressure of unknown cause is still little known

PURPOSE OF THE STUDY

High blood pressure is associated with ischaemic heart disease cerebrovascular disease and renal disease which are important causes of morbidity and mortality in Sweden

The prevalence of high blood pressure in Sweden is unknown (14) In hospital series the blood pressure readings are those of special aggregations who because of highly selective mechanisms cannot be deemed representative of anything but themselves The blood pressure of a random sample of subjects living in urbanisation areas has not been studied in any detail whatsoever The first objective therefore was to obtain the prevalence of high blood pressure in men living in Goteborg Sweden of city with a population of 412 545 (1963) The ratio of untreated to treated hypertensive subjects was of particular interest

The purpose was also to study extreme groups of the blood pressure distribution —high blood pressure and low blood pressure in which investigations as to causative factors could be concentrated With an adequate identification group characteristics as to obesity diseases heart volume, biochemistry and socioeconomic status could then be examined The aim of this was to find associations or correlations between two sets of events which could lead to an hypothesis that could then be tested by experiments Another question of interest was whether diastolic blood pressure is better related to the various manifestations of hypertension than systolic

The study of men born in 1913 was divided into two parts

I a cross sectional study

II a longitudinal study

The present work is based only on the first part The first follow up examination of these men will be done during the year 1967

CHARACTERISTICS OF THE STUDY POPULATION

Sweden shares the same latitude as Alaska and Greenland, but the Gulf Stream makes the climate more agreeable In Göteborg, the highest and the lowest temperatures of the year are 27° and −20° and the mean temperature 6 9°C The yearly rainfall is 860 mm, the mean relative humidity 71 per cent and the mean atmospheric pressure 1014 millibars (150) The city is on the western coastline facing the Skagerack and the open Atlantic, and is the second largest in Sweden It is a port, but also the centre of an industrial region with shipyards, a car industry, a ballbearing factory and engineering firms

Sweden is organised into seven medical regions and each region has a regional hospital with full facilities in all specialities Sahlgrenska sjukhuset in Goteborg, where the study took place, is the hospital for the region It treats also all acute diseases in the city

There is national compulsory sickness insurance in Sweden Inpatient hospital treatment is free, but the patients pay a proportion of the cost for outpatient consultation A social insurance gives the patient a reasonable income when he is ill All employers and self employed people in Sweden above 16 years of age receive a daily sickness allowance when they report an illness Morbidity studies were compiled from the information of the General Sickness Insurance Office in Goteborg

Goteborg 1913–1963

During the years 1913–1963, the size and the composition of the population of Goteborg has changed (Table 1) The total population increased 142 per cent The population of individuals less than 15 years of age increased about 65 per cent while for those over the age of 65 the

TABLE 1 Population in Goteborg by sex and various ages 1911–1963

Year	Total Population	Population under 15 years		Population over 65 years	
		Male	Female	Male	Female
1911	170 606	25 162	23 968	3 346	6 212
1920	202 328	27 468	26 574	4 203	7 576
1930	243 414	26 663	25 937	6 281	10 162
1940	281 287	25 224	24 258	7 715	11 642
1950	353 687	39,134	37 130	12 425	17 023
1960	404 349[1])	44 004	41 313	18 303	24 639
1963	412 545[2])	41 961	39 248	20 4[illegible]6	27 571

[1]) 1 XI 1960
[2]) 1 XI 1963

TABLE 2. Overcrowded persons in Goteborg 1910–1960 (more than 2 individuals per room excluding kitchen)

Year	Per cent overcrowded persons
1910	64
191	59 9
19 0	65 0
1930	55 8
1940	42 1
1945	38 1
1950	31 9
1955	25 5
1960	18 3

TABLE 3 Passenger cars (private vehicles) registered in Goteborg 1943–1963 per 1000 inhabitants

Year	Cars per 1000 inhabitants
1944	19 8
1947	20 8
1950	27
195	36 5
1953	43 8
1954	56 3
1956	85 9
1958	116 3
1960	144 1
1961	155 9
196	1 0 0
1963	18 3

increase was 510 per cent for males and 344 per cent for females

From official statistics it was possible to elucidate some of the socio economic changes The percentage of overcrowded persons (defined as living quarters with more than 2 persons per room exclusive of kitchen) had decreased from 65 per cent in 1910 to 18 per cent in 1960 (Table 2) The number of private automobiles had steadily risen from 19 8 cars in 1944 to 187 3 cars per 1000 habitants 1963 (Table 3) There had also been important changes in the death rate as shown by Table 4 For males and females aged 40 there was a decrease from 7 65 and *6 39 to 1 46 and 1 66* respectively The decrease was less for those aged 50 while at the age of 60 years there was no decrease from 1946–1950 for males but *there was still a decrease for females*

Procedure

The population to be studied was selected by the Revenue Office Goteborg from their register which by law must be kept up to date The sample consisted of the entire population of men who were born in 1913 on those days which were even multiples of three (e g 3 6 9 etc) and who were living at that time in the

TABLE 4 Death rate at age 40 50 and 60 by sex 1911 1960 (per 1000 in age group)

	40 years		50 years		60 years	
	Men	Women	Men	Women	Men	Women
1911 19 0	65	6 39	11 96	9 09	28 05	16 99
19 1 1930	5 39	4 84	12 30	9 61	19 03	17
1931 1940	4 55	3 18	9 50	7 99	22 49	17 65
1941 1945			—		—	—
1946–1950	9	2 46	8 9	[illegible]	16 89	12.03
1951 19 5	3 05	2 06	5 48	4 60	16 0	11 33
1956–1960	1 46	1 66	6 51	4 5	16 45	9

city of Goteborg The sample was selected twice, once in May 1962 and then the final sample in November 1962, this was so as to be able to collect in advance the participant's records and x ray reports from the hospitals in Goteborg

Various steps were taken to encourage subjects to participate in the study The Trade Union Movement, the Employers General Association, the City authorities of Goteborg and the industries of the city were contacted All those consulted approved the idea of the examination and they gave it their wholehearted support during its progress Thus many of the participants could attend the examination without financial loss for the time involved

The persons selected were invited by a letter to a combined population study and health examination on a specified date The letter explained the aim of the study, and it stressed that, if reliable results were to be obtained, it was essential that all individuals should attend If a subject did not present himself for examination, the author went to see him personally and tried to persuade him to come

A series of trial examinations of medical students and volunteers was conducted a week before embarking on the main study The date of the examination of the participants was determined by their place of employment, starting at the beginning of the year with the largest industries

The study continued throughout the whole of 1963

The examination lasted 4 hours starting at 7 15 a m and took place in the out patient department, Medical Service I, Sahlgrenska sjukhuset

Response to examination

Of the 973 subjects selected, 855 were investigated at the Sahlgrenska sjukhuset (hospital group), and 38 were studied in their home (home group) Two were interviewed by telephone and are included in the home group Seventy eight did not take part in any form of examination (non participant group) (Table 5) An analysis of the non participant group has been reported (154)

It was originally planned also to carry out a full examination of the individuals in the home group However, it was soon realized that the subjects were influenced by the visit of the author For example the pulse rate of the first subjects in the group were seldom below 120 and from their behaviour, they were often thought to be upset The examination at home included therefore only a questionnaire concerning chronic bronchitis angina pectoris, previous illnesses, social and economic factors and the subjects weight and height

TABLE 5 Examination status of study sample of 973 men aged 50 living in Goteborg

	Number	%
Sample drawn Nov 7 1962 by Revenue Office	973	100
Examined or interviewed		
Examined at the Sahlgrenska sjukhuset (Hospital group)	855	88
Interviewed in Participant's Home (Home group)	40	4
Non Participants¹)	78	8

¹) Includes refusals (24) failed to trace (32) deceased (7) left Goteborg (9) admitted to hospital (4) other reasons (2)

Place of birth Education and Civil status

Information concerning place of birth and civil status for all the sample was obtained from registers of the Revenue Office

In table 6 the places of birth for the hospital group home group and non participant group are presented 73 per cent of the hospital group were born in Goteborg or the surrounding counties The corresponding figure for the non participant group was 57 per cent

Education is shown in table 7 The subjects in the hospital group and the home group were asked about their education Information on current occupation was obtained from the registers of the Revenue Office The subjects in the non participation group were counted as having high school education or more if it was clear that their occupation required such It is evident that there were no important differences in education level

In table 7 the civil status is also presented There were great differences here In the hospital group 85% were married compared to 53% in the home group and 40% in the non participant group

TABLE 6 Place of birth for study sample of 973 men aged 50 living in Goteborg

	Goteborg		Surrounding counties[1]		Rest of Sweden		Foreign countries		Not known	
	n	%	n	%	n	%	n	%	n	%
Total sample (n 973)	443	46	256	26	219	23	53	5	2	—
Hospital group (n 855)	400	47	222	26	187	22	44	[illegible]	[illegible]	—
Home group (n 40)	16	40	16	40	8	20	0	—	0	—
Non participant group (n 78)	27	35	18	23	24	31	9	11	0	—

[1] Goteborg and Bohus Halland Älvsborg and Skaraborg

TABLE 7 Education and civil status in study sample of 973 men aged 50 living in Goteborg

	Higher education		Married		Divorced		Widower		Unmarried	
	n	%	n	%	n	%	n	%	n	%
Total sample (n 973)	157	16	779	80	69	9	10	1	107	11
Hospital group (n 855)	144	17	[illegible]	85	51	6	3	0 4	74	9
Home group (n 40)	5	12	21	53	16	15	1	2	12	30
Non participant group (n 78)	8	10	31	40	2	28	6	8	22	28

Pension

Twenty three men in the total sample were registered at the General Sickness Insurance Office in Goteborg as receiving invalid pensions Table 8 shows the number of subjects with mental and somatic diseases The majority of the cases was found in the hospital group (15/23) For the somatic diseases all cases were examined or interviewed

Earlier cardiovascular diseases

From the records collected prior to the study, it was possible to obtain information about previous diseases The number of persons with previous cardiovascular disease in each of the three groups is presented in table 9 There was no over representation in the home group or in the non participant group of persons with regard to previous cardiovascular disease

The home group was asked about chest pain, angina pectoris and myocardial infarction The questionnaire used is described in more detail later in this work (page 12) Chest pain was recorded for 6 subjects (15%) in the home group and 176 subjects (21%) in the hospital group There was one case of angina pectoris and one of myocardial infarction among the 40 in the home group, which corresponds well with prevalence in the hospital group (2 1 and 0 9 per cent, respectively)

Causes of death 1950–1962

It was possible to obtain the death certificates from the Central Bureau of Statistics, Stockholm for all male subjects born in 1913 and registered as dying in Goteborg between 1950–1962 Before 1950 another classification system was used The causes of death in the younger age

TABLE 8 Number of men with an invalid pension in study sample of 973 men aged 50 living in Goteborg

	Mental diseases	Somatic diseases	Total
Total sample ($n=973$)	13	10	23
Hospital group ($n=855$)	6	9	15
Home group ($n=40$)	1	1	2
Non participant group ($n=78$)	6	0	6

TABLE 9 Number of men treated in hospital for cardiovascular disease prior to examination in 1963 among Study Sample of 973 men aged 50 living i Goteborg

	Myocardial infarction	Cerebrovascular diseases	Hypertensive diseases	Total
Total sample ($n=973$)	10	3	7	20
Hospital group ($n=855$)	8	2	5	15
Home group ($n=40$)	1	0	0	1
Non participant group ($n=78$)	1	1	2	4

TABLE 10 Income in a study sample of 9 3 men aged 50 living in Goteborg

	Income unknown		10 000 Sw Kr		10 000–19 000 Sw Kr		20 000 Sw Kr	
	n	%	n	%	n	%	n	%
Total sample (n=9 3)	48	(4 9)	166	(17 1)	565	(58 0)	194	(0 0)
Hosp tal group (=855)	33	(3 9)	121	(14)	5 0	(60 8)	181	(1)
Home group (n=40)		(5 0)	1	(30 0)		(55 0)	4	(10 0)
Non part c pant gro p (n=78)	13	(16)	33	(42 3)	3	(9 5)	9	(11 5)

groups is also less relevant to the problem studied The causes of death for the 33 subjects born on the same days as the series selected are presented in Fig 1 For the years 1950–1958 (the subjects ages were 37–45 years) accidents and suicides predominated In the last five years before the examination year 1963 the pattern changed there were 9 cases of neoplasms and for the first time vascular diseases such as ischaemic heart disease and cerebral haemorrhage (5 cases) appeared

Income

Information about the income in 1961 of the selected men and their wives was acquired from the Revenue Office in Goteborg

From table 10 it is clear that the subjects in the home and non participation groups were more often found in the low income group (income less than 10 000 Sw Kr) and less often seen in the highest group (income equal to or more than 20 000 Sw Kr) There were also more

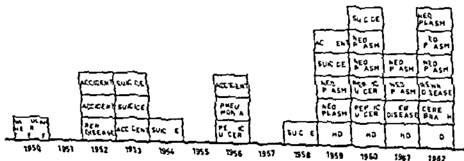


Fig 1 Causes of death in 33 men born in 1913 on the same days as the selected series (Goteborg 1950–6) (IHD = Ischaemic heart disease)

subjects in these groups for whom no information (in most cases the same as no income) could be obtained from the registers

In the hospital group, 43 per cent of the subjects had wives who had an income. In the home groups, 23 per cent, and in the non participant group, 18 per cent had wives with income. The income level of the wife was about the same in the three groups

Weight and height

The subjects in the home group were asked about their weight and height. In table 11 the results are compared with the measurements made on subjects in the hospital group. The home group was slightly heavier and taller although the standard deviations are similar

Discussion

The death rate curves emphasize the rapid increase with age

In studying groups of subjects with wide age range (for example men age 40–60), a man of 40 has a very small chance of dying from diseases like arteriosclerosis, cancer, hypertension compared to a man of 60. A man of 40 has lower blood pressure (85), and his relative weight and median subscapular skin *fold thickness* are lower compared to older men (109)

A large number of changes have been observed in the function of human organ systems and in homeostatic mechanisms with increasing age. Practically all of the homeostatic mechanisms which have been studied show a decline in functional capacity. The decline usually has its onset shortly after maturity and is progressive into old age (89). As has been showed by Strehler (117) there is a very marked age related deterioration in function of the cardiovascular, respiratory and renal systems

Most of the factors with which this study is concerned are related to age. In order to standardize the important influence of ageing, the simplest way appears to be a study of individuals of the same age

In this series of men born in 1913, the mean age when they were examined was 49 years and 11 months (range 49 years, 1 month–50 years, 11 months. S D 5.1 *months)*. This range could have been reduced if the subjects had been investigated closer to their date of birth but this was not practicable

The environment of Sweden has changed rapidly during the last 20 years. A man of 50 in 1963 has lived a life which has differed in several ways from the life of a man of 50 in 1943. This is illustrated by

TABLE 11 Height and weight in study sample of Men aged 50 living in Goteborg

	Weight (kg)	S D	Height (cm)	S D
Hospital group ($n=855$)[1]	75 9	11 0	175 0	6 0
Home group ($n=39$)[2]	77 4	12 6	176 5	6 3

[1]) Data missing for 4 subjects

[2]) Data missing for 1 subject

the figures for overcrowded persons and number of cars An effective way to reduce the influence of such secular changes seems to be to study persons of the same age

To permit valid conclusions the sample studied must be representative of its parent population A non participation group of 10% of the total sample may sometimes jeopardize the interpretation of the results and othertimes be of less importance depending on the frequency of the conditions studied and the extent of bias in the sample

The analysis of the non participant group in this study showed that its members differed in income and civil status They were also registered more frequently in the Register of Temperance Board (154) suggesting the presence of alcoholic problems These factors must be born in mind when interpreting the influence of social economic factors on health

The aim of this study was to investigate blood pressure It is therefore important to determine if the non participant group was biased with regard to blood pressure or associated conditions The blood pressure itself is not measured but the proportion of subjects with a history of cardiovascular diseases was not increased in the non participant group Height and weight in the home group were similar to those of the hospital group The greatest difference between the three groups was in the socio-economic factors (see p 70)

The non participant group amounts to only 8% of the total sample so even if it was very different this would not greatly affect the conclusions In fact the differences between the total group and the hospital group were in general small (tables 6—10)

A cross sectional study has several drawbacks Illness or other characteristics can mostly be ascertained only for the time of the examination The number of persons affected at a single point in time depends upon the difference in the rate at which new cases arise (incidence) and the rate at which established cases are removed (recovery rate+mortality rate+rate of removal for other reason) A disease that has a high mortality rate will have a lower prevalence rate than another with the same incidence rate but lower mortality rate The spontaneous recovery rate of hypertensive disease is unknown For rate of removal the most important factor must be migration to and from Goteborg The size of the group which moved from Goteborg after the examination has not yet been ascertained but the group which moved to Goteborg between May and November 1962 shows the same blood pressure distribution as the remainder

It is important to find out the cause of death for those men born in 1913 but dying before the examination in 1963 since their characteristics may have differed from those of the survivors who were actually examined

The commonest causes of death were neoplams accidents and suicides Of special interest are the number of deaths from kidney diseases (2) cerebral haemorrhage (1) and ischaemic heart disease (4) All of these conditions may be associated with high blood pressure but since only 7 cases are involved the selective bias introduced by their removal cannot have been great

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A drawback to the method used in this study is that age differences concerning high blood pressure cannot be studied, but the vast amount of available data on cross sectional studies on age differences must be considered adequate and there seems to be little need for further studies of the subject

On the other hand the influence of age on blood pressure in middle age and later life cannot be investigated in a cross sectional study (138) In the older subjects mortality rises with increasing blood pressure and hence a single population survey gives a misleading impression of age trends Those who survive will have lower mean pressures than those who die and the age trend in a cross sectional study will understate the rise of pressure with age in a cohort of individuals who live through the years A better way to study the influence of age on blood pressure is to follow a cohort This will be included in part II of this study With such an approach the fact that all of the individuals investigated are born in the same year must be an advantage

Time table of an examination day

Every weekday except in the period from the 15th of June to the 15th of August 1963 4–6 subjects came for examination They arrived at 7 15 a m in the fasting state During the first 45 minutes they passed four examination stations At the first station blood and urine samples were taken at the second station an E C G was recorded and at the next station the author interviewed all subjects about their work social and environmental background and symptoms of chronic bronchitis and angina pectoris At the last station anthropometric measurements were made At approximately 8 a m an x ray of the heart and lungs was taken by the author and heart volume in prone position was measured by a technician After a light breakfast (coffee and sandwiches) all subjects took part in an interview about previous diseases then they received a physical examination and measurement of blood pressure By now the time was 9–10 a m and about 10 o'clock the subjects went to the department of ophthalmology where an ophthalmologist examined the eyes

Questionnaire

The questionnaire used by the author for social data smoking diseases and family history also included a questionnaire about cardiovascular symptoms which has been designed and tested at the London School of Hygiene and Tropical Medicine (145) A Swedish translation of this was used

Blood pressure measurements

Recommendations from W H O have been followed (7)

The author measured the blood pressure of all subjects with the same mercury manometer on each occasion between 9–10 a m in the same room where noise and chilling were avoided After 5–10 minutes talking with the man sitting in a chair a cuff with a rubber bag which was 12 cm wide and 23 cm long was applied firmly and evenly to the right arm The lower edge of the cuff was 2 cm above the antecubital space The subject's arm was supported in extension and abduction The cuff was quickly inflated to 200 mm Hg the stethoscope applied to the edge of the cuff cuff pressure permitted to fall at a rate of 2–3 mm Hg per pulse beat and the point at which the pulse beat was first audible was read The cuff pressure was allowed to go on falling and the point of muffling (phase 4) and the point at which the sound disappeared (phase 5) were recorded When the systolic sound was audible immediately after inflation the cuff pressure was allowed to fall down to zero and a fresh inflation to 250 mm performed The blood pressure was read to the nearest 5 mm Hg more precise readings were considered to be of little value The first systolic reading was recorded No correc-

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Minnesota code (16) without access to other information about the subjects.

Heart volume

The heart volume was determined in the standing position by the method described by Jonsell (74). Radiological examination of the heart was made in all cases by the author. It started with a fluoroscopy to obtain information about the lungs and the configuration of the heart. The frontal film was exposed at deep inspiration. For the lateral films the subject stood with the forearms crossed over his head keeping the chest as vertical as possible. The exposure was made after a barium swallow to obtain contrast of the oesophagus. The tube film distance for both positions was 1.5 m.

The following three diameters were read by the two roentgenologists (S. Paulin M.D. and S. Johannesson M.D.): the long diameter (L) from the junction between the aorta and the right atrium to the left lower pole, the broad diameter (B) from the right basal border of the cardiac silhouette to the junction between pulmonary conus and left ventricle and the sagittal diameter (D) from the posterior contour of the sternum to the oesophagus. Jonsell's calculation of the heart volume is based upon the formula $V = k \cdot L \times B \times D$. The constant k is found to be 0.4.

Heart volume was also determined in the prone position with two plane roentgengrams using the method of Larsson and Kjellberg (91) as modified by Kjellberg, Rudhe and Sjöstrand (89). In this method the subject lies face down with the central rays directed transversely from the right to obtain the sagittal plane and for the frontal plane directed 30° caudally in relation to the vertical direction to obtain a modified transverse picture with the lower profile of the heart free from the diaphragm. The pictures were taken in planes that are at right angles to each other, but they were not taken simultaneously and were without relation to E.C.G. and heart cycle. The same technician took the pictures except in a few cases.

Humerfelt (70) has shown that the method of estimating the heart size seems profoundly sensitive to observer variation when the subject is investigated in the standing position. In order to evaluate whether the two observers differed in calculating the heart volume, both of them trained roentgenologists, the following experiments were undertaken.

40 randomly selected pairs of films from assessment of heart volume in prone position were read independently by the two observers. Standard error for difference was 6.2 ml which was not significant ($t = 0.36$).

From the left oblique position in the x-ray of the heart a qualitative estimation of hypertrophy of left ventricle was done.

The roentgenologist had no information about the history of the subjects.

Blood investigations

At 7.15 a.m. a nurse drew blood samples from an antecubital vein without stasis. Four nurses were engaged in blood drawing during the study in rotation.

The analysis were made in a research laboratory with experienced personnel (Medicinska Undersökningscentralen, Sahlgrenska sjukhuset). Serum was obtained by allowing the blood to coagulate for

tions for arm circumference have been made in this study During part of the study, arm circumference was measured in 96 consecutive subjects and the median value was found to be 28 cm Two arms were less than 24 cm and one more than 35 cm If the correction table recommended by Pickering (132) had been used, this would have caused no change or at most only a slight change in the majority of subjects

Eye ground examination

One ophthalmologist (Elisabeth Aurell, M D) carried out ophthalmoscopy of all subjects during the whole study She had no information about the history of the subjects The examination was made on the same day as the rest of the study and at the same time, i e 10–11 a m A Comberg ophthalmoscope was used and the investigation was made on the direct as well as the inverted image of the fundus For every subject the presence or absence of the following eye ground changes was recorded,

1) general attenuation of arterioles
2) focal narrowings of arterioles
3) haemorrhage
4) exudate
5) oedema
6) widening of light reflex of the arterioles
7) crossing phenomena
8) occlusion of vessels

Anthropometric measurements

The anthropometric measurements were done by the same observer (Hans Hjortzberg Nordlund, M D) during the whole study The subjects were dressed in undershorts only The observer measured body weight to the nearest 0 1 kg, using a lever balance The height was measured barefooted with the subjects heels, buttocks, shoulders and head touching the wall The head was level, with the eyes looking straight ahead

The fat was studied by measurements of skinfold thickness with a Harpenden caliper (45) The pressure applied by the caliper was 10 g/mm² and the area of the contact surface was 20 mm² The skin and subcutaneous tissue were gripped with finger and thumb 1 cm above the point of measurement and squeezed firmly The skinfold was measured to the nearest 0 1 mm

Skinfold measurements were taken at three different sites on the right side of the body 1) The lateral thorax over the lower ribs, midway between the axilla and the iliac crest—the subject in supine position 2) The abdomen to the right of the umbilicus—the subject in supine position 3) The back just below the inferior angle of right scapula Three estimations were made from each side and the average skinfold thickness in mm was used for the calculations

Electrocardiographic examination

The subject was placed in a supine position on a couch A trained nurse took the E C G after the subject had been resting for about 10 minutes A direct writing recorder (Mingograf 42 Elema Schonander AB Sweden) was used and three leads were recorded simultaneously A paper speed of 50 mm/sec was used Leads I, II III, aVR aVL aVF, V1 V2 V4 V5 V7 were recorded The electrocardiograms were interpreted by the author using the

Minnesota code (16) without access to other information about the subjects

Heart volume

The heart volume was determined in the standing position by the method described by Jonsell (74). Radiological examination of the heart was made in all cases by the author. It started with a fluoroscopy to obtain information about the lungs and the configuration of the heart. The frontal film was exposed at deep inspiration. For the lateral films the subject stood with the forearms crossed over his head keeping the chest as vertical as possible. The exposure was made after a barium swallow to obtain contrast of the oesophagus. The tube film distance for both positions was 1.5 m.

The following three diameters were read by the two roentgenologists (S. Paulin M.D. and S. Johannesen M.D.): the long diameter (L) from the junction between the aorta and the right atrium to the left lower pole, the broad diameter (B) from the right basal border of the cardiac silhouette to the junction between pulmonary conus and left ventricle and the sagittal diameter (D) from the posterior contour of the sternum to the oesophagus. Jonsell's calculation of the heart volume is based upon the formula $V = k \cdot L \times B \times D$. The constant k is found to be 0.4[illegible].

Heart volume was also determined in the prone position with two plane roentgengrams using the method of Larsson and Kjellberg (91) as modified by Kjellberg, Rudhe and Sjöstrand (88). In this method the subject lies face down with the central rays directed transversely from the right to obtain the sagittal plane and for the frontal plane directed 30° caudally in relation to the vertical direction to obtain a modified transverse picture with the lower profile of the heart free from the diaphragm. The pictures were taken in planes that are at right angles to each other but they were not taken *simultaneously* and were without relation to E.C.G. and heart cycle. The same technician took the pictures except in a few cases.

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Armitage (3) was used The test is a modification of χ^2 test All the data from the subjects were fed into a Computer (SAAB D21) for Statistical analyses of the results

Discussion

In the present study of blood pressure the epidemiological method has been used which limits the techniques available for investigating the subjects The advantages of the method become clear however, when it is compared with those used in the study of hospital series

A *basic principle* in research is that a sample must be chosen in such a way that it is possible to extend conclusions from it to its parent population Therefore, a method of either random or stratified sampling is used This basic principle is the main reason that an epidemiologist studies a random sample of subjects from a society and tries to identify a group of ill subjects This group of subjects can then be considered representative of all those with the disease in the whole population

In contrast clinical investigators in their studies of hospital patients are usually unable to take into account that their sick subjects are highly selected frequently in a way that biases their conclusions

Clinical investigators often use a group of subjects (blood donors hospital staff medical students firemen other patients) as controls for a sample of patients under study It is impossible to be sure that all relevant factors are matched Volunteers may differ in some important respect e g temperament sickness social class from the general population or the population under study and are therefore not suitable as controls In a study of a random sample from a population it is possible to choose representative controls

When blood pressure is the variable studied observer variation diurnal changes and seasonal changes are important Lowe and McKeown reported a substantial systematic inter observer variation in recording blood pressure (96) There were mean differences between readings by different doctors of as much as 15 mm Hg The level at which an observer reads systolic pressure gives little information about the level at which he reads diastolic pressure In the study of Keys and collaborators great discrepancies were noted among observers in Greece, Finland Dalmatia and Slavonia (85)

The diurnal changes are important in most variables studied here Kain Hinman and Sokolow (78) measured blood pressure with a portable recorder in hypertensive subjects and found that the average pressure between 8 and 11 a m was significantly greater than the average pressure after 11 a.m ($p<0.002$ for systolic blood pressure and $p<0.01$ for diastolic blood pressure) Bock and Kreuzenbeck (18) found for 13 normotensives that the lowest blood pressure occurred during the night between 1 a.m and 5 a m The maximum pressures were recorded in the morning and in the evening Richardson Honour Fenton Stott and Pickering reported that systolic and diastolic pressures in healthy subjects were significantly higher in the late afternoon and evening than in the morning, and fell to lowest values during the early hours of sleep rising again as the subject woke up (141)

Renbourn (140) and Cranston and Brown (33) reported diurnal decrease of

about 2 hours at room temperature (18–20°) All analyses, except for uric acid, were made on the same day that the subjects were examined

Cholesterol was determined according to Theorell as revised by Cramer and Isaksson (32)

Triglycerides were determined according to the method of Carlsson and Wadstrom (27) The triglycerides were expressed as glyceride glycerol and given in mMoles/l The glyceride glycerol values show a tendency towards a skewed distribution, as pointed out by Carlsson (26) The statistical analysis has therefore been carried out after conversion to a logarithmic scale to normalise the distribution

Fasting blood sugar was determined by use of glucose oxidase, peroxidase and O dianisidine (Reagent from AB Kabi, Stockholm) (92)

Venous hematocrit Blood was taken without stasis into tubes containing 100 IU dry heparin (AB Vitrum, Stockholm) A micro hematocrit method was used with capillary tubes (Wifug, Stockholm) (length=50 mm, diameter of capillary 0 8 mm) The speed of the centrifuge was 3000 r p m and the duration of spinning 15 minutes The mean value of two duplicates was used in the calculations

Uric acid was determined according to Praetorius (136) using Beckman DU spectrophotometer from frozen serum

Blood sedimentation rate in one hour was determined by Westergren s method The test was done within 1 hour of the blood being drawn

Urine osmolality As soon as the participants arrived in the morning, a urine sample was taken No instructions were given them about fluid restriction the evening before or the morning of the examination Determination of urine osmolality is described elsewhere (157)

Proteinuria As a qualitative test for urine protein a dip stick method (Ames, U S A) was used

Plasma creatinine was determined according to Technicon Auto Analyzer

Electrophoresis of serum protein The serum protein fractions were analysed by paper electrophoresis in TRIS buffer, pH 8 9, according to Aronsson and Grönvall (6) The total serum protein was determined by the Biuret method The investigations were performed on the day that the subjects were examined All analyses of serum proteins were made by the same person

Statistical methods

Conventional statistical methods were used for calculation of mean value and standard deviation Comparison of mean values of different groups of subjects was made by Student s t test Conventional probability levels of significance have been used

When the number of subjects with a sign or affection in the extreme blood pressure groups S_1D_1[1]) and S_4D_4 were small the total series were divided in B P I (S_1D_1 S_2D_1 S_1D_2), B P II (S_2D_2) B P III (S_2D_3 S_3D_2 S_3D_3) and B P IV (S_3D_4 S_4D_3, S_4D_4 and treated subjects) In order to answer the question whether the frequencies show a significant trend upwards or downwards with increase in height of blood pressure a test for linear trends in proportions and frequencies described by

[1]) For explanation of the symbols see p 28

range of 25–30 mm) and relative to the variation between individuals In a survey of 722 working men who had been examined annually in four successive years Armitage et al (4) reported that the standard deviation representing the random differences between readings for an individual subject was estimated to be 9 1 mm for systolic pressure and 7 2 mm for diastolic pressure They pointed out that the variance of single readings of blood pressure in many population studies has been attributed entirely to differences between individuals without consideration of the possibility that an important component arise from variability within individual subjects

In spite of the spontaneous variation in blood pressure Harlan et al (65) believed that a single determination of this variable may serve to place the subject within a certain relative grouping They reported a tendency for individuals to maintain their relative ranking within the same group on serial examinations

The value of casual blood pressure is thus limited It is therefore desirable to study other manifestations of hypertensive disease in order to classify an individual

Study of the retinal vessels in the hypertensive subject provides a unique opportunity to observe directly arteriolar changes in the vascular system In the fundus oculi the small arteries and arterioles are visible to direct inspection and by ophthalmoscopy it is possible to obtain information about vasoconstriction of these arterioles

Keith Wagener and Barker (83) divided hypertensive retinopathy into four degrees of severity and showed that these correlated closely with the prognosis in the untreated patient The diagnosis of most severe categories (Grades III and IV) depends on the presence of exudative changes, such as flame shaped haemorrhages cotton wool spots, hard exudates and papill oedema The two milder grades (Grades I and II) are diagnosed by the presence of narrowing and irregularity of arterioles thickening of the walls with increase in light reflex, and changes at arteriovenous crossings In this classification so called arteriolosclerotic retinal vessel changes (thickening of the walls and crossing phenomenon) are not separated from the hypertensive or angiospastic ones A better method is to study the various phenomena in the eye grounds separately (8) In the present study two signs have been recorded as manifestations of hypertensive disease namely, focal narrowings of arterioles and general attentuation of arterioles Irregularity of calibre with focal narrowing is one of the most common features of retinal arterioles in the hypertensive patient Dollery (40) has found that the great majority of these changes persist even if hypertension was apparently cured by renal surgery The pathogenesis of the narrow segments remains a problem but the lesions seen in the eye of a subject with established hypertension appear to be fixed and represent structural changes in the vessel wall (40)

hematocrit from the morning to late evening Mayer (105) found also a decrease of hematocrit during the day, but his number of subjects was small and the change was not significant Diurnal changes in blood sugar and triglycerides are well known

Diurnal variations in E C G have been registered both in unipolar precordial leads and standard extremity leads (79)

The time of the day also affects heart volume measurement (168)

Many biological functions evidently show diurnal variations They have received little attention and for the most part have not been taken into consideration in other population studies

Seasonal variations in blood pressure in men have been reported by Rose (144) and Paul *et al* (125) Rose found a peak in the spring and a trough in the late summer Paul *et al* found the highest blood pressure in the winter and lowest in the summer The differences however, were small in both studies

For practical reasons, in the present study it was impossible within a short time to investigate near 1000 men and also take into consideration the effect of diurnal changes The influence of season is in some way reduced because of the pause in the investigations during the summer

In this study, as in all population studies the casual blood pressure is recorded It was dictated by the necessity to have a convenient method which was not time consuming Those who use casual blood pressure often underestimate the effects of its variability Richardson (1964) *et al* reported when studying the diurnal changes in blood pressure that, in more than half of the hypertensive patients, the pressure fell to levels that would be accepted as entirely normal on a casual day time reading (141) Hinman, Engel, and Bickford (1962) obtained data on normotensives which showed considerable variations during the day, and these variations could be related to events in the everyday life of the subject (67) The validity of a single casual blood pressure has been questioned for a long time Freis (1960) states that casual blood pressure may not be representative of the average blood pressure to which a subject s cardiovascular system is subjected He has pointed out that the difference between casual and basal blood pressure varies over a wide range in different individuals that in some subjects they are close, and in others wide apart, some subjects may be hypertensive in the casual reading and normotensive in the basal readings (58)

On the other hand, Richardson *et al* (141), in their study of diurnal changes in blood pressure found that basal blood pressure were never as low as those recorded during sleep with the automatic blood pressure recorder Indeed, they were often close to the highest pressures recorded with the apparatus They doubted the superiority of a basal blood pressure over a single casual pressure in epidemiological work

Armitage and Rose (5) have discussed how much reliance can be placed upon a single estimation of blood pressure In a laboratory study of 10 subjects during a 6 week period and without standardising the time of examination each subject showed a variability which was large, both on an absolute scale (a

procedure for blood pressure measurement When the studies with the most similar procedure of measurement were compared (Evans County Framingham, Tecumseh and Goteborg) the differences are considerably reduced

Our data based on 855 men aged 50 showed no evidence for bimodality in the distribution of either systolic or diastolic blood pressure It has been noted several times before that blood pressure in the general population shows a distribution that is skewed to the right This skewing is more marked for systolic than for diastolic blood pressure It is clear from figure 1 that this sample of men of Goteborg aged 50 follows the general pattern

TABLE 13 Mean systolic and diastolic blood pressure in men aged 50

Sitting	N	Mean	S D
Systolic blood pressure	855	138	20 0
Diastolic blood pressure			
Phase 4	855	92	13 2
Phase 5	855	89	13 0

Classification of subjects according to blood pressure

One possibility for classifying subjects is to use different systolic or diastolic levels of blood pressure Table 15 illustrates the difficulty of such a method If subjects are grouped by classes of diastolic blood pressure there will be a wide scatter of systolic blood pressure among classes and vice versa For example in the group with 105 mm Hg diastolic pressure the systolic blood pressure varied from 115 mm Hg to 190 mm Hg On the other

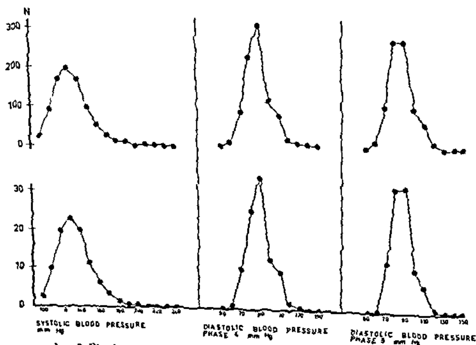


Fig 2 Blood pressure in men aged 50 (n=855) Frequency distribution curves

BLOOD PRESSURE DISTRIBUTION CLASSIFICATION OF SUBJECTS

The distribution of blood pressure in a population may be compared between communities and can be of importance in explaining different cardiovascular, cerebrovascular and renal mortality rates

The recent sharp differences of opinion about the etiology of essential hypertension is concerning the presence of uni or bimodal distribution of arterial blood pressure in communities (130) (131) (133) (134)

Results

Table 12 presents the rules for assessment of blood pressure and the conditions present when the blood pressure was taken in different studies

The distribution curve for systolic blood pressure and diastolic pressure (phase 4 and 5) in the sitting position, both in absolute numbers of men and in percentage in the present study, is given in Fig 2 Table 13 shows the mean systolic and diastolic blood pressure Table 14 compares mean systolic and diastolic blood pressure in this series, with seven other population studies of white men

The range of the mean values of the systolic blood pressure in these eight studies was 132 6–148 mm for the age group 45–54 The range of the mean values of diastolic blood pressures was 79 3–91 6 The data from the present study agree quite well with the majority of the studies The systolic blood pressure in the present study was lower than that in the majority of the others and the diastolic blood pressure was higher than that in the majority of the other studies The slight differences between the studies can be largely explained by the lack of a standard

TABLE 12 Rules and conditions for blood pressure measurement in eight population studies

	Posture	Time of day	Environment	Diastolic phase	Number of observers	Number of readings after which the blood pressure was measured
Muskogee (U S)	Sitting	All times	Subject s home (91%)	5	9	3
Evans County (U S)	Sitting	All times	Special clinic	5	2	1
Framingham (U S)	Sitting	All times	Special clinic	[illegible]	Several	2
Tecumseh (U S)	Sitting	All times	Hospital	[illegible]	Several	1
Finland	Sitting	All times	Health locals	5	Several	[illegible]
Bergen (Norway)	Sitting	All times	Schools	4 or 5	Several	2 3
Ronda Fach (Wales)	Sitting	All times	Subject s home	4	1	1
Goteborg	Sitting	9–10 a m	Hospital	4 and 5	1	1

TABLE 15 Distribution of subjects in different blood pressure groups ($n = 841$)[1]

Systolic B P	50	55	60	65	70	75	80	85	90	95	100	105	110	115	120	125	130	135	140	145	150
S_4 240																	1				
235																					
230														1						1	
225																			2		
220															1						
215														1							
210															2		1				
205																					1
200													1		1	1					
195														1		1					
190										1	1	1	2		2	2					
185											1	1		3	1			1			
180												1	1	3		1					
175											1	2	[illegible]	4	1						
S_3 170									1	2	1	3	4	2		1					
165									1	4	2	3	4	2							
160							1	2	3	5	6	2	10	3	2	1					
155							1	3	5	12	5	6	5	1							
150						1	2	3	13	19	14	8	5	1	1						
S_2 145						1	4	5	18	24	10	7	7	1							
140					1	1	7	15	26	14	11	7	5								
135					1		9	16	29	24	8	2									
130					1	5	14	17	34	16	6		3	1							
125					2	4	15	24	19	11	3										
120		1		1	8	10	27	14	17	3	1										
115			1		11	6	12	9	4	1		1									
S_1 110			1	2	10	6	9	5	3												
105				3	5	4	3	1													
100				1	1	3															

←— D_1 —→ (50–70) ←— D_2 —→ (75–95) ←— D_3 —→ (100–110) ←— D_4 —→ (115–150)

Diastolic B P (Phase 4)

[1] Subjects with antihypertensive treatment were excluded

A suitable extreme group large enough for statistical handling consists of about 5 per cent of the total sample of these men in both directions The cut off values for the extreme groups of systolic blood pressure were 175 mm Hg and 110 mm Hg and for diastolic blood pressure 115 and 70 mm Hg The reading of blood pressure in 5 mm intervals did not permit exact grouping into percentiles

In table 16 the total sample of men is grouped into four systolic (S_{1-4}) and four diastolic blood pressure groups (D_{1-4}) according to the rules above The 14 sub

hand in the group with 160 mm Hg systolic blood pressure the diastolic blood pressure varied from 80 to 125 mm Hg. This lack of uniformity within blood pressure classes gives heterogeneous groups and reduces the information about the combined influence of the two blood pressures on the men.

The variability of blood pressure in an individual subject increases the difficulty of a correct classification and justifies broad grouping. The extreme groups—high blood pressure and low blood pressure, are of particular interest. The variability of the blood pressure in the extreme groups has not been systematically elucidated, but McKeown *et al* (101) reported from a follow up of a population three to four years after first arterial pressures were recorded, that about 85% of the readings of systolic pressures were found to be within ±20 mm Hg of the original reading, while diastolic pressures were within the range of ±20 mm Hg in 97% of the subjects. In spite of the fact that most of the changes in the extreme groups may regress towards the mean value of the distribution, all the subjects in these groups would be likely to differ from each other and in most cases from one intermediate group.

In this classification the systolic blood pressure and diastolic blood pressure (phase 4) was used. The subjects were first ranked according to their blood pressure. The upper quartile contains the 25 per cent of subjects with the highest blood pressure. For systolic blood pressure the upper quartile cut-off value in this group of men happens to be at 150 mm Hg and for diastolic blood pressure at 100 mm Hg. These two values are the first dividing lines employed in the analysis.

TABLE 14 Systolic and diastolic blood pressure from eight population studies of white males

	Muskogee (U S)	Evans County (U S)	Framingham (U S)		Tecumseh (U S)	
	(45–54 years)	(45–54 years)	(45–49 years)	(50–54 years)	(45–49 years)	(50–54 years)
Systolic blood pressure	132.6	142.9	138.9	141.0	141	148
Diastolic blood pressure (Phase 5)	86.1	91.6	88.2	88.6	88	88

	Finland West		Finland East		Bergen (Norway)	
	(45–49 years)	(50–54 years)	(45–49 years)	(50–54 years)	(45–49 years)	(50–54 years)
Systolic blood pressure	133.9	138.9	144.2	149.9	134.3	138.7
Diastolic blood pressure (Phase 5)	79.3	81.2	87.6	89.4	84.8	86.4

	Rhonda Fach (U K)		Goteborg (Sweden)
	(45–49 years)	(50–54 years)	50 years
Systolic blood pressure	134.2	137.5	138
Diastolic blood pressure (Phase 5)	83.1	86.1	89

TABLE 17 Distribution of subjects according to different blood pressure combinations

	D_1 (≤ 70)	D_2 (75–95)	D_3 (100–110)	D_4 (≥ 115)
S_4 (≥ 175)		1	14	33
S_3 (150–170)		71	78	14
S_2 (115–145)	29	468	71	2
S_1 (≤ 110)	26	34		

sex and age group As Pickering has pointed out dips in frequency distribution curves occur by chanse when numbers are small (130) From our data it is clear that in a large general population sample of men of the same age there is no sign of bimodality in the distributions

Humerfelt has recently reported on a new Bergen Study of a 10 per cent sample (8272 persons) of the adult population in which they have succeeded in recording the blood pressure in 98 3 per cent of the selected group (71) In this large sample the blood pressure distribution shows no tendency at all to bimodality nor is there any bimodality when this population is split into different sex and age groups Humerfelt concludes that the bimodality of the frequency distribution curves presented in earlier series is an artefact attributable to such factors as small numbers in the series and measurement errors

Classification of subjects according to blood pressure

Before considering the significance of deviation in blood pressure in a population we must find some cut off values in the distributions which give optimal splitting between cases and controls The problem is not easy to solve The continuity of the systolic and diastolic blood pressure curve is well known and suggests that it is in fact impossible on any grounds to find a single cutting off point on the curve, which clearly distinguishes two classes of subjects

In spite of that the usual approach is to take some arbitrary point on the curve and call the subjects above that point abnormal or hypertensive This is not defensible on statistical grounds Another complication with this system is the heterogeneity of the groups defined Use of a classification system with 160 mm Hg as the lowest pathological systolic value and/or 95 mm Hg as the lowest diastolic level will produce from this sample of men classes of subjects whose blood pressure varies from 115/95 to 230/145 It is difficult to see the point of such a classification

Another defect of this approach is that it does not distinguish between systolic and diastolic hypertension There appears to be no appreciation of the possibility that a high systolic with normal or slightly elevated diastolic pressure may well reflect merely the existence of arteriosclerosis of the aorta In the present study both systolic and diastolic blood pressure were divided into 4 groups and every subject of the sample placed in one of 10 combinations of systolic and diastolic blood pressure

jects receiving antihypertensive treatment have been separated from the others in the analysis Their casual blood pressure level on therapy is not representative for themselves Treatment may also influence other hemodynamic and biochemical factors analysed In some population studies the treated cases are included in the whole series, but there is no apparent justification for such an approach

In order to reduce the heterogeneity of the groups, a combination of the level of both systolic and diastolic pressure has been used in the present investigation (tables 15 and 17)

Of the 16 possible combinations of blood pressure, most interest was focused on the two extreme groups S_1D_1 (≤ 110 and ≤ 70) and S_4D_4 (≥ 175 and ≥ 115) These two groups were compared with S_2D_2 (115–145/75–95), which was considered as an intermediate blood pressure group and from which controls matched by height, weight and skinfold thickness were drawn (Appendix)

When the number of subjects with a sign or affection in the extreme groups was small the groups were combined in the following way B P I (S_1D_1, S_2D_1, S_1D_2) B P II (S_2D_2) B P III (S_2D_3 S_3D_2, S_3D_3), B P IV (S_3D_4, S_4D_3, S_4D_4 and treated subjects)

In group S_4D_2 there is one subject and in S_2D_4 only two subjects These three men are excluded from further analysis

Discussion

Blood pressure distribution

There was a close correlation between the mean values of systolic and diastolic blood pressure obtained in places as different as Evans County, South Wales and Goteborg Oldham *et al* have pointed out the similarity in mean pressure values between another set of studies in Britain farm workers in the U S A and tea workers in Assam, India (118)

Such evidence would suggest but not conclusively establish that whatever differences in environment may be found between the populations studied they are not likely to be important in the determination of mean blood pressure level

The finding of the unimodality in the distribution of systolic and diastolic blood pressure agrees with the result from most population studies with large age ranges

The blood pressure distributions cited here consist of data from persons of different ages, and it is known that the blood pressure distribution changes with age When this factor is taken into consideration the numbers are often small in each

TABLE 16 The distribution of subjects according to systolic and diastolic blood pressure

	Systolic B P				
	S_1 (≤ 110)	S_2 (115–145)	S_3 (150–170)	S_4 (≥ 175)	Total
Number	60	570	163	48	841[1]
	Diastolic B P				
	D_1 (≤ 70)	D_2 (75–95)	D_3 (100–110)	D_4 (≥ 115)	Total
Number	55	574	163	49	841[1]

[1]) Subjects with antihypertensive treatment were excluded

TABLE 17 Distribution of subjects according to different blood pressure combinations

	D_1 (≤70)	D_2 (75–95)	D_3 (100–110)	D_4 (≥115)
S_4 (≥175)		1	14	33
S_3 (150–170)		71	78	14
S_2 (115–145)	29	465	71	2
S_1 (≤110)	26	34		

sex and age group As Pickering has pointed out dips in frequency distribution curves occur by chance when numbers are small (130) From our data it is clear that in a large general population sample of men of the same age there is no sign of bimodality in the distributions

Humerfelt has recently reported on a new Bergen Study of a 10 per cent sample (8272 persons) of the adult population in which they have succeeded in recording the blood pressure in 98 3 per cent of the selected group (71) In this large sample the blood pressure distribution shows no tendency at all to bimodality nor is there any bimodality when this population is split into different sex and age groups Humerfelt concludes that the bimodality of the frequency distribution curves presented in earlier series is an artefact attributable to such factors as small numbers in the series and measurement errors

Classification of subjects according to blood pressure

Before considering the significance of deviation in blood pressure in a population we must find some cut off values in the distributions which give optimal splitting between cases and controls" The problem is not easy to solve The continuity of the systolic and diastolic blood pressure curve is well known and suggests that it is in fact impossible on any grounds to find a single cutting off point on the curve, which clearly distinguishes two classes of subjects

In spite of that the usual approach is to take some arbitrary point on the curve and call the subjects above that point abnormal or hypertensive This is not defensible on statistical grounds Another complication with this system is the heterogeneity of the groups defined Use of a classification system with 160 mm Hg as the lowest pathological systolic value and/or 95 mm Hg as the lowest diastolic level will produce from this sample of men classes of subjects whose blood pressure varies from 115/95 to 230/145 It is difficult to see the point of such a classification

Another defect of this approach is that it does not distinguish between systolic and diastolic hypertension There appears to be no appreciation of the possibility that a high systolic with normal or slightly elevated diastolic pressure may well reflect merely the existence of arteriosclerosis of the aorta In the present study both systolic and diastolic blood pressure were divided into 4 groups and every subject of the sample placed in one of 10 combinations of systolic and diastolic blood pressure

Before answering questions regarding the prevalence of high blood pressure, the *phenomenon under consideration* must be defined. Is it possible to define it solely *on the* basis of quantitative criteria? Murphy has pointed out a fashion, which cannot be too strongly condemned, of cutting off the end of a distribution curve, endowing it with some pretentious name beginning with 'hyper' and ending with 'emia' or 'osis' and then devoting much effort to seeking the cause (113).

Instead of selecting a cutting off point, which for some reason suits the investigator, there is another approach to definition. It is defensible to use some point *as a dividing line on the blood* pressure distribution curve if the group above this level can be identified by some biological characteristic independent of the blood pressure. An attempt, therefore, has been made here to study the relationship *of clinical manifestations of hyper*tensive disease *other than blood* pressure itself, to different levels of the systolic and diastolic blood pressure.

There is at least one group of subjects having high blood pressure, which is easy to define and which has not been systematically studied previously in population *studies—subjects under antihypertensive* treatment, although this depends upon the criteria usually used for treatment.

In connection with defining high blood pressure the following phrase was mentioned in an editorial in the British Medical Journal (1959) (48). Some, however, may feel that in a study *of hyper*tension which by definition is diastolic, little would be lost, and some embarrassment possibly saved, if for purposes of classification, *the systolic pressure could* be ignored'. There is a common impression among physicians working in clinical medicine that diastolic blood pressure is a more stable and important measure than systolic blood pressure, although the population studies which have investigated the reproducibility and prognostic value of the two pressures do not support this hypothesis (101), (167). The influence of systolic blood pressure and diastolic blood pressure on the organism can be evaluated from this sample of men aged 50 in which the age factor having been eliminated and several manifestations of hypertensive disease having been studied. It is possible therefore, to decide whether high systolic blood pressure is as innocent as widely *believed in clinical medicine*.

Blood pressure and manifestations of high blood pressure in eyegrounds and hearts

The following definitions for manifestations of high blood pressure have been used here: hypertensive eye ground changes (focal narrowing and general attenuation of arterioles). ECG findings (high R amplitude and ST—T changes, Minnesota Code III 1, IV 1-3, V 1-3) *left ventricular hypertrophy*—LVH (sign of hypertrophy of left ventricle in quali

tative estimation from the left oblique position in the x ray of the heart)

Table 18 and figure 3 present the frequency of manifestations of high blood pressure in different blood pressure groups. In the low blood pressure groups (S_1D_1, S_1D_2, S_2D_1) there were no eye ground changes, a low frequency of ECG findings and LVH (x ray). The percentage of findings for all variables was greatest in S_4D_4. In this group most subjects (30/33) had one or more of the manifestations studied.

The single most characteristic sign of high blood pressure among those considered here appeared to be focal narrowings in the arterioles of the eye ground. In S_4D_4 the frequency of this finding was 64.0 per cent compared to 0.6 per cent in S_2D_2.

It was also clear that S_3D_4 and S_4D_3 with highest diastolic blood pressure only $\geq$115 and highest systolic blood pressure $\geq$175 respectively had less manifestations of hypertension when compared with S_4D_4. In these two groups 20/28 had one or more manifestations of high blood pressure which was significantly less than in S_4D_4 (χ^2=3.88 D F $p<0.05$) where the subjects had both the highest diastolic blood pressure ($\geq$115) and systolic blood pressure ($\geq$175).

On the basis of the findings presented here the group S_2D_2 seems to be suitable as a control group for S_4D_4 and S_1D_1. The blood pressure was of intermediate order and the frequency of eye ground changes low.

Subjects under antihypertensive treatment

There were 14 subjects (1.6 per cent) who at the time of examination were

TABLE 18 Prevalence rate of manifestations of high blood pressure in different blood pressure groups (in per cent)

	S_1D_1 (n=26)	S_1D_2 (n=31)	S_2D_1 (n=50)	S_2D_2 (n=408)	S_2D_3 (n=71)	S_3D_2 (n=71)	S_3D_3 (n=78)	S_3D_4 (n=14)	S_4D_3 (n=14)	S_4D_4 (n=33)	Antihypertensive treatment (n=14)
SBP mm Hg	100–110	100–110	115–145	115–145	115–145	150–170	150–170	150–170	175+	175+	
DBP mm Hg	70	75–95	70	75–95	100–110	75–95	100–110	115+	100–110	115+	
Focal narrowings of the arterioles (eye ground)	0	0	0	0.6	2.8	2.8	0.0	7.1	35.0	64.0	71.4
Attenuated arterioles (eye ground)	0	0	0	1.7	5.6	7.0	20.5	50.0	50.0	58.0	93.0
ECG findings (Minnesota Code III 1 IV 1–3 V 1 3)	15.4	8.8	3.4	23.8	38.0	31.0	34.0	50.0	29.0	40.0	30.0
LVH (x ray)	19.0	5.9	0.0	7.5	5.6	15.4	12.8	7.1	35.7	49.0	35.7

3

receiving antihypertensive drugs (the treated group) Their actual blood pressure and information about hospitalization are given in table 19 All subjects were under some form of diuretic treatment Only five subjects had been hospitalized

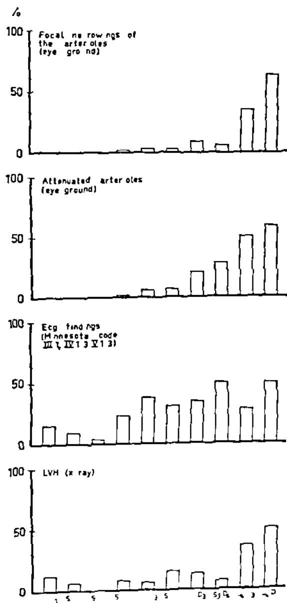


Fig 3 Frequency of manifestations of high blood pressure in different blood pressure groups

TABLE 19 Blood pressure in subjects with antihypertensive treatment ($n=14$)

Case no	S B P mm Hg	D B P mm Hg (phase 4)	Hospitalized
35	170	125	—
84	175	85	—
135	155	110	—
188	185	115	—
192	130	85	—
257	190	130	—
301	190	120	+
482	175	105	—
582	170	105	—
729	195	150	+
786	160	110	—
873	140	105	—
893	180	130	—
964	175	115	—

Only three had been under treatment in the same medical clinic

One subject of the 14 treated had had papilloedema severe heart failure and other signs of malignant hypertensive disease in 1957 At the time of examination (1963) he was in better condition working a full day without papilloedema but with a plasma creatinine of 3 7 mg per 100 ml

Of interest was the comparison of the treated group with S_4D_4 and S_2D_4 (table 20) It was apparent that S_4D_4 and the treated group were similar in blood pressure and in frequency of manifestations

Four subjects had been under antihypertensive treatment but stopped for various reasons At the time of examination they fell into blood pressure groups S_1D_2 S_3D_2 S_4D_3 S_4D_4 The man in S_1D_2 also had pulmonary tuberculosis and reduced lung function

Prevalence of high blood pressure

The prevalence rate of high blood pressure in 841 men aged 50 according to various criteria is presented in table 21 14 subjects under antihypertensive treatment were excluded There was a wide variation from 1 7 per cent to 41 5 per cent in prevalence according to these commonly used criteria The most popular criterion for hypertension nowadays is systolic blood pressure 160 and/or diastolic pressure 95 (7) The prevalence rate for this class is 41 5 per cent When systolic blood pressure ≥175 and diastolic blood pressure ≥115 were used as dividing lines a group of subjects was found in which most of them (30/33) had other manifestations of high blood pressure On the basis of this the group S_4D_4 ($n=33$) was defined as high blood pressure When this group was added to the group under antihypertensive treatment ($n=14$) a prevalence rate of high blood pressure of 5 [illegible] per cent was found Rates from other classification systems may be estimated from table 15

Systolic vs diastolic blood pressure

A comparison in prevalence rate of different manifestations of high blood pressure between systolic and diastolic blood pressure is found in figure 4 and the corresponding data are given in tables 22 and 23

The frequency of manifestations rises in a similar way with rise in systolic and diastolic blood pressure The number of subjects in each of the systolic and diastolic groups was roughly equal The group S_4 had higher frequency rate for all manifestations except LVH (x ray)

A comparison between S_4D_3 and S_3D_4 and between S_3D_2 and S_2D_3 can be of

TABLE 21 Prevalence of high blood pressure in 841 men aged 50 according to different criteria[1])

	Per cent
D B P 115+ mm Hg (134)	1 [illegible]
S B P 180+ mm Hg and D B P 110+ mm Hg (34)	3 8
S B P 175+ mm Hg and D B P 115+ mm Hg	3 9
S B P 160+ mm Hg and/or D B P 95+ mm Hg (7)	41 5

[1]) Subjects with antihypertension treatment were excluded

TABLE [illegible] Comparison of frequency of hypertensive manifestations between subjects in S_3D_3, S_4D_4 and subjects with antihypertensive treatment

	n	Systolic B P	Diastolic B P	Eye ground changes: Focal narrowings	Eye ground changes: *Attenuated* arterioles	ECG findings: Minnesota *Code III 1* IV 1–3 V 1–3	*LVH* x ray
S_3D_3	4[illegible]8	130 4	87 0	0 6	1	7 5	[illegible]3 [illegible]
S_4D_4	33	196	12[illegible]	64 0	[illegible]8 1	43 0	43 0
Antihypertensive treatment	14	1[illegible]0	113 6	[illegible]1 4	93 0	36 0	3[illegible] [illegible]

receiving antihypertensive drugs (the treated group) Their actual blood pressure and information about hospitalization are given in table 19 All subjects were under some form of diuretic treatment Only five subjects had been hospitalized

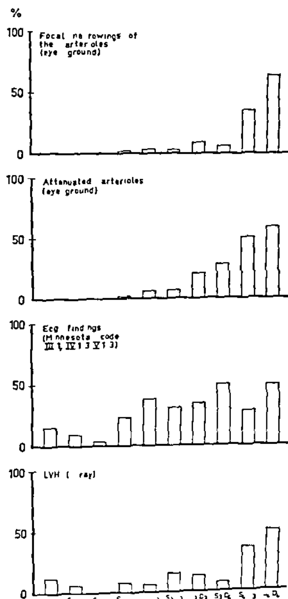


Fig 3 Frequency of manifestations of high blood pressure in different blood pressure groups

TABLE 19 Blood pressure in subjects with antihypertensive treatment ($n=14$)

Case no	S B P mm Hg	D B P mm Hg (phase 4)	Hospitalized
35	170	125	–
84	175	85	+
135	155	110	–
188	185	115	–
192	130	95	–
257	190	130	–
351	190	120	–
482	175	105	–
582	170	105	–
729	195	150	–
786	160	110	–
873	140	105	+
893	160	130	–
964	175	115	–

Only three had been under treatment in the same medical clinic

One subject of the 14 treated, had had papilloedema, severe heart failure, and other signs of malignant hypertensive disease in 1957 At the time of examination (1963) he was in better condition working a full day, without papilloedema but with a plasma creatinine of 3 7 mg per 100 ml

Of interest was the comparison of the treated group with S_4D_4 and S_-D_2 (table 20) It was apparent that S_4D_4 and the treated group were similar in blood pressure and in frequency of manifestations

Four subjects had been under antihypertensive treatment but stopped for various reasons At the time of examination they fell into blood pressure groups S_-D_2 S_3D_- S_4D_3 S_4D_4 The man in S_2D_4 also had pulmonary tuberculosis and reduced lung function

pressure groups On the other hand manifestations of cardiac involvement were found up to 23 per cent in S_2D_4 and in the lower blood pressure groups The explanation is that ECG findings and LVH (x ray) are less specific signs of high blood pressure For example ECG items Minnesota Code IV 1-3 and V 1 3 are also signs of coronary heart disease

It is often difficult to decide whether a subject with an elevated casual blood pressure should be treated However a casual blood pressure of 175/115 or greater in a subject in the age group studied meant that there were almost always other concomitant manifestations of high blood pressure and that specific therapy must be considered

Several recent American studies have determined the prevalence rate of high blood pressure In the Framingham study 10 per cent of the subjects had a systolic pressure exceeding 160 mm Hg and in 33 per cent the diastolic pressure exceeded 86 mm Hg (35) Among the entire population of Tecumseh Michigan about 30 per cent of the men and 34 per cent of the women had pressures of 160/95 or greater (51) In the Health Examination Study U S A of white men aged 45-54 18 3 per cent had a blood pressure of at least 160 systolic and/or 95 diastolic (61)

TABLE 93 Prevalence rate of manifestations of high blood pressure in different diastolic blood pressure groups

	D_1 < [illegible]0 (n [illegible]5[illegible])	D_2 [illegible]-95 (n = 574)	D_3 100-110 (n = 163)	D_4 > 115 (n = 49)
Focal narrowings of the arterioles	0%	0 9%	8 6%	4 8%
Attenuating arterioles	0%	[illegible] 4%	16 6%	46 9%
ECG findings)	5 5%	8 5%	11 [illegible]%	[illegible]6 [illegible]%
LVH (x ray)	9 1%	3 [illegible]%	35 6%	48 9%

) Minnesota Code III 1 IV 1 3 V 1 3

TABLE [illegible]4 Comparison of frequency (absolute numbers of hypertensive manifestations between the group S_1D_4 and the group S_2D_4

	S_1D_4 (n = 14)	S_2D_4 (n = 14)
Focal narrowings (eye ground)	7	1
Attenuating of arterioles (eye ground)	5	4
Ecg findings)	5	1
LVH (x ray)	4	7

Minnesota Code III 1 IV 1 3 V 1 3

TABLE [illegible]5 Comparison of frequency (absolute numbers) of manifestations of high blood pressure between S_1D_3 and S_2D_3

	S_1D_3 (n = [illegible]1)	S_2D_3 (n = [illegible]1)
Focal narrowings (eye ground)	[illegible]	[illegible]
Attenuating arterioles (eye ground)	[illegible]	4
Ecg findings)	11	4
LVH (x ray)	[illegible]2	[illegible]

) Minnesota Code III 1 IV 1-3 V 1 3

interest (tables 24 and 25) With these it is possible, in a rough way, to study the separate effect of systolic blood pressure from diastolic blood pressure and vice versa The number of subjects in the group was too small to justify a statistical analysis but there is a definite suggestion that apart from LVH (x ray) all the other manifestations have a closer relationship to a high systolic blood pressure

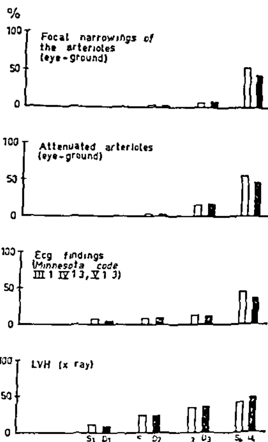


Fig 4 Frequency of manifestations of high blood pressure in systolic blood pressure groups S_1–S_4 (white columns) and in diastolic blood pressure groups D_1–D_4 (black columns)

Discussion

When systolic blood pressure 175 mm Hg and diastolic blood pressure 115 mm Hg were used as dividing lines on the blood pressure distribution curve, a group (S_4D_4) was *found in which it was possible* to identify most of the subjects (30/33) by other manifestations of high blood pressure These cutoff points seemed to be suitable for this sample of men in order to get a conceptual group of high blood pressure In group S_2D_2 there were found few subjects with focal narrowings and attenuated arterioles in the eyeground *(3/468 and 8/468)* This group was considered suitable as a control group for S_4D_4 and S_2D_2

The relationship between eye ground changes and blood pressure differed from the relationship between ECG findings *and LVH (x ray) and blood pressure* Eye ground changes were seen very seldom in S_2D_2 and never in the lower blood

TABLE 22 Prevalence rate of 4 manifestations of high blood pressure in different systolic blood pressure groups

	S_1 ≤110 (n = 60)	S_2 115–145 (n = 570)	S_3 150–170 (n = 163)	S_4 175 (n = 48)
Focal narrowings of the *arterioles*	0%	0 9%	6 1%	54 2%
Attenuated arterioles	0%	2 1%	15 3%	50 2%
ECG findings[1])	8 3%	7 0%	13 5%	45 8%
LVH (x ray)	11 -%	24 2%	34 4%	41 %

[1]) Minnesota Code III 1 IV 1–3 V 1–3

hospital when it is complicated and hence not representative of the subjects with high blood pressure in the population as a whole Robinson's finding (143) illustrates another mechanism of selection He showed that individuals who had high blood pressure and who attended a hospital showed more neurotic symptoms than those with similar blood pressure who did not attend hospital He discussed the process of going to hospital and suggested that the practitioner takes part in the selection of hospital cases Amongst the patients coming to him with various symptoms he will find some with high blood pressure He then has to decide whether to treat these patients himself or to send them to hospital It seems reasonable to suppose that those who were sent to hospital would be those who had symptoms the doctor could not explain or those whose illness had not responded to treatment or those who were just difficult in some way Findings of deviations of laboratory examinations such as serum cholesterol must influence his decision Also many neurotic symptoms (headache palpitations) may lead the practitioner to take blood pressure so that he may discover a high blood pressure that would in the absence of neurotic symptoms have remained unrecognized

Few studies have been concerned with the question of antihypertensive treatment in representative populations The prevalence rate of this condition depends on two factors namely the prevalence of high blood pressure and the extent of medical care In Baldwin County Georgia (165) 41 per cent of hypertensive persons were unaware of their hypertension only 30 per cent of the known hypertensives were under treatment The control of blood pressure was judged to be adequate in not more than 14 per cent of all the known hypertensives It is, of course difficult to know how adequate the treatment is from a single blood pressure measurement but from table 19 it is clear that the majority of the treated subjects in the present study still had a rather high casual blood pressure

In this age group elevated systolic blood pressure was as much associated with hypertensive cardiovascular manifestations as elevated diastolic blood pressure When the independent effects of the two blood pressures were compared there was a trend towards higher frequency of ECG findings and eye ground findings according to systolic blood pressure, and a trend toward higher frequency of LVH (x ray) according to high diastolic blood pressure

The inter relationships between electrocardiographic abnormalities and physical characteristics were examined by Keys *et al* in their large total population study They found that all positive associations of ECG abnormalities with arterial blood pressure were more distinct with the systolic than with the diastolic blood pressure levels (85)

It is important to realize that in this sample of men the number of subjects with what J Conway has defined as arteriosclerotic hypertension was very low (30) There is just one man who has a diastolic blood pressure not exceeding 105 mm Hg and pulse pressure at least equal to the diastolic pressure It is possible that at older ages the relationship between systolic pressure and hypertensive manifestations will be weaker be

All these studies indicate that high blood pressure defined according to the blood pressure value is exceedingly common in United States

It is difficult to make comparisons as to the number of subjects with hyper tension in different localities Epstein has shown that small differences in popula tion may matter greatly if one uses arbitrary cutoff points such as 160 mm Hg systolic blood pressure (50) Tech nical differences in measuring blood pres sure are also important From table 12 it is clear that the rules of blood pressure assessment are not the same for all the studies compared In the Health Exami nation Study study (61) for example, the average of three measurements was ob tained for each individual In the present work and in many other population stu dies, only one measurement was used It is known that on the average the first three readings show a progressive fall (38) Rose has found regularly a fall in systolic blood pressure but a small rise in diastolic blood pressure (146) A further complication in making comparisons is that systematic observer variation can cause important differences in blood pres sure distributions as has been shown by Lowe and McKeown (96)

In the present study there were found 33 subjects with blood pressure 175/115 or greater and further 14 subjects under antihypertensive treatment The preva lence rate of high blood pressure defined according to these more specific criteria at the age of 50 can be assessed as 5 5 per cent Of interest is the comparison of the prevalence rate of high blood pressure with those of other diseases found in this group of men aged 50 (table 26) Peptic ulcer and high blood pressure were most common in these men

In this study for every treated case of hypertensive disease there were more than two untreated and of the 14 treated cases only five had been hospitalized This indicates that of the 47 subjects with hypertensive disease in the population sample, only 10 per cent would have appeared in a hospital study of the di sease Most people are suspicious about findings from population studies, which have a participation rate of 70 per cent or less The mechanisms of selection are apparent and the study sample is biased

There are, for several reasons, great differences between hospital series of sub jects with high blood pressure and the subjects with high blood pressure in a community Only a small fraction of all hypertensive subjects comes to the hos pital for investigation This provides an op portunity for uncontrolled mechanisms of selection Among these personal and socio economic factors may be involved A patient with two serious diseases is more likely to seek attention in hospital than a patient with one disease Hypertension is therefore more likely to be seen in

TABLE 26 Prevalence rate of some diseases in men aged 50 ($n=855$)

	Per cent
Angina pectoris (155)	2 1
Myocardial infarction (hospitalized) (155)	0 9
Diabetes mellitus (under treatment) (9)	0 8
Chronic bronchitis with obstruc tion (155)	1 8
Peptic ulcer with symptoms (156)	5 6
High blood pressure	5 5

To date practically all population studies concerned with the effect of body build on morbidity and longevity have been investigations of relative body weight (weight for given height) to the study variables The weight of the human body is made up of a number of components, bone viscera body fluids muscle tissue and fat Obviously overweight may be accounted for by a heavy skeleton, by a great mass of muscle or by a large accumulation of fat As Keys has said (84) the last two components muscle and fat are in many ways metabolic opposites and have entirely different significance Unfortunately to date little information on even one component of body composition is available on a sufficiently large number of individuals in any given population group to study its relationship to morbidity and mortality

Schweitzer *et al* (147 a) in a review found that the association of overweight and high blood pressure has been noted in several studies but they considered the results thus far inconclusive Geiger and Scotch (60) write Obesity has long been believed to be associated with high blood pressure but whether this is due to real differences in actual blood pressures or to an artifact in the measurement of blood pressure related to the cuff size of the sphygmomanometer is not clear" Taylor (1965) concluded that the available information does not support the idea that the deposition of adipose tissue causes the development of high blood pressure (62)

There is an obvious need for separating obesity and overweight The relationship of obesity to blood pressure has been studied in a representative population sample and the results are presented in this chapter

The influence of arm size on blood pressure assessment is important Some author (15) consider that the association found between overweight and blood pressure can be explained by the artifact of too high auscultatory blood pressure reading obtained in fat individuals Of interest therefore is the relation of other hypertensive manifestations to obesity If they show a relationship this is quite separate from the influence of arm size on blood pressure measurement

Results

Subscapular skinfold thickness, weight and height

Significant differences were found between S_1D_1 and S_4D_4 in subscapular skinfold thickness and weight compared to the control group S_2D_2 (table 27) S_1D_1 had less mean value of skinfold thickness and weight and S_4D_4 had higher mean values of skinfold thickness and weight There were no significant differences in height between the groups

Fat hypertensive subjects vs non fat hypertensive subjects

In order to compare the fat hypertensive subjects to the non fat hypertensive

cause of the usual loss of elasticity of the aorta and larger arteries Of interest is a study by Roso (146) of systolic and diastolic blood pressure levels in 'severe hypertension" in a clinical series He concluded that the data provided no support for the conventional view that the malignant phase of hypertension is more closely linked with diastolic than with systolic hypertension If anything they slightly favour the reverse

nine groups using the 33 and 66 percentiles of subscapular skinfold thickness and weight as dividing lines. The prevalence rate of eye ground changes are presented in table 29. In table 30 the mean value of systolic blood pressure, diastolic blood pressure, subscapular skinfold and weight for the nine combinations are shown.

In subjects with light weight (<70.8 kg) there was no increase in rate of eye ground changes with rise in skinfold thickness. In lean subjects (subscapular skinfold <12.1 mm) there was no increase in prevalence of eyeground changes with rise in weight. On the other hand in the heavy subjects (≥79.5 kg) there was an increase with rise in skinfold thickness (χ^2=6.[illegible]2, 1 D F, p<0.0[illegible]). The fat subjects (subscapular skinfold ≥16.7 mm) showed also an increase in rate of eye ground changes with rise in weight (χ^2=7.03, 1 D F, <0.01). A modification of χ^2 test was

TABLE 29 Prevalence rate of hypertensive eye ground changes in different combinations of weight and subscapular skinfold thickness

Subscapular skinfold (mm)	Weight (kg) <33 percentile	Weight (kg) ≥33 percentile–<66 percentile	Weight (kg) >66 percentile
>66 percentile	6.9% (n=29)	8.3% (n=72)	20.9% (n=18[illegible])
≥33 percentile–≤66 percentile	5.6% (n=[illegible])	9.1% (n=13[illegible])	13.9% (n=[illegible]9)
<33 percentile	6.0% (n=182)	6.3% (n=[illegible]9)	0.0% (n=22)

TABLE 30 Blood pressure, weight and skinfold thickness in subjects in different combinations of weight and skinfold thickness

Subscapular skinfold		Weight <33 percentile	Weight ≥33 percentile–<66 percentile	Weight ≥66 percentile
66 percentile	S B P mm Hg	13[illegible]	136	147
	D B P mm Hg	87	9[illegible]	9[illegible]
	S F mm	[illegible]0.6	19.3	[illegible]3.[illegible]
	Weight kg	66.[illegible]	[illegible]5.1	90.1
33 percentile–<66 percentile	S B P mm Hg	13[illegible]	140	142.3
	D B P mm Hg	8[illegible]	9[illegible]	94.3
	S F mm	13.9	14.3	14.8
	Weight kg	66.4	[illegible]	84.6
<33 percentile	S B P mm Hg	130	13[illegible]	131
	D B P mm Hg	66	90	88
	S F mm	8.9	10.0	10.6
	Weight kg	63	74.3	83.3

subjects concerning other manifestations of high blood pressure the subjects in B P IV (S_3D_4, S_4D_3, S_4D_4) was divided into fat (≥18 4 mm subscapular skinfold) and non fat (<18 4 mm subscapular skinfold) using upper quartile value of subscapular skinfold as a dividing line (table 28) In all groups, the fat hypertensive subjects had a higher frequency of other manifestations of high blood pressure The results showed that the fat hypertensive subjects had as advanced or more advanced high blood pressure than the non fat hypertensive subjects

Prevalence of fat subjects

The number of fat subjects (subscapular skinfold thickness ≥18 4 mm) in S_1D_1 ($n=26$), S_2D_2 ($n=466$) and S_4D_4 ($n=32$) were 19 per cent, 22 per cent and 52 per cent, respectively This indicates that there were in this sample a considerable number of fat subjects with low blood pressure and intermediate blood pressure

Hypertensive eye ground changes and subscapular skinfold thickness and weight

Hypertensive eye ground changes (focal narrowings and/or attenuated arterioles) were used as manifestations of high blood pressure independent of arm size The total series was divided into three groups using the 33 and 66 percentiles of subscapular skinfold thickness and weight as dividing lines The prevalence of eye ground changes in these groups are presented in table 29 and 30 There was a significant rise in rate of eye ground changes with increase in subscapular skinfold and weight

The total series was then divided into

TABLE 27 Comparison of subscapular skinfold thickness weight and height between subjects in different blood pressure groups

	S_1D_1 $n=26$	S_2D_2 $n=468$	S_4D_4 $n=33$
Subscapular skin fold (mm)	11 8	14 7[3])	19 9
S D	4 6	5 3	9 1
P[1])	<0 01		<0 001
Weight (kg)	68 4	75 2[2])	82 9[2])
S D	9 6	9 9	15 4
P[1])	<0 001		<0 001
Height (cm)	173 8	175 2[2])	175 2[3])
S D	7 4	6 0	6 5
P[1])	n s		n s

[1]) The differences S_1D_1–S_2D_2 and S_4D_4–S_2D_2 were tested

[2]) Data from two subjects were missing

[3]) Data from one subject was missing

TABLE 28 Comparison between fat hypertensive subjects and non fat hypertensive subjects in B P [1]) IV (S_3D_4 S_4D_3 S_4D_4) with reference to other manifestations of high blood pressure in per cent

	B P IV (S_3D_4 S_4D_3 S_4D_4)	
	Fat subjects S F ≥ 18 4 mm $n=28$	Non fat subjects S F < 18 4 mm $n=33$
Focal narrowings	61%	46%
Attenuating arterioles	57%	33%
Ecg findings (Minnesota Code III 1 IV 1–3 V 1–3)	29%	18%
LVH (x ray)	57%	33%

[1]) Subjects with antihypertensive treatment were excluded

nine groups using the 33 and 66 percentiles of subscapular skinfold thickness and weight as dividing lines. The prevalence rate of eye ground changes are presented in table 29. In table 30 the mean value of systolic blood pressure, diastolic blood pressure, subscapular skinfold and weight for the nine combinations are shown.

In subjects with light weight (<70.8 kg) there was no increase in rate of eye ground changes with rise in skinfold thickness. In lean subjects (subscapular skinfold <12.1 mm) there was no increase in prevalence of eyeground changes with rise in weight. On the other hand in the heavy subjects (≥79.5 kg) there was an increase with rise in skinfold thickness ($\chi^2=6.52$, 1 D.F., $p<0.0[illegible]$). The fat subjects (subscapular skinfold ≥16.[illegible] mm) showed also an increase in rate of eye ground changes with rise in weight ($\chi^2=7.09$, 1 D.F., $p<0.01$). A modification of χ^2 test was

TABLE 29 Prevalence rate of hypertensive eye ground changes in different combinations of weight and subscapular skinfold thickness

Subscapular skinfold (mm)	Weight (kg) <33 percentile	≥33 percentile–<66 percentile	≥66 percentile
>66 percentile	6.9% (n = [illegible]9)	8.3% (n = [illegible])	[illegible]0.9% (n = 18[illegible])
>33 percentile–<66 percentile	5.6% (n = [illegible])	9.1% (n = 13[illegible])	13.9% (n = 9)
<33 percentile	6.0% (n = 18[illegible])	6.3% (n = 9)	0.0% (n = [illegible])

TABLE 30 Blood pressure, weight and skinfold thickness in subjects in different combinations of weight and skinfold thickness

Subscapular skinfold		Weight <33 percentile	≥33 percentile–<66 percentile	≥66 percentile
66 percentile	S.B.P. mm Hg	13[illegible]	136	147
	D.B.P. mm Hg	8[illegible]	9[illegible]	99
	S.F. mm	[illegible]0.6	19.3	[illegible]3.5
	Weight kg	66	[illegible]1	90.1
33 percentile–<66 percentile	S.B.P. mm Hg	135	140	14[illegible].3
	D.B.P. mm Hg	88	9[illegible]	94.3
	S.F. mm	13.9	14.3	14.8
	Weight kg	66.4	[illegible]	84.6
<33 percentile	S.B.P. mm Hg	13[illegible]	13[illegible]	131
	D.B.P. mm Hg	86	90	88
	S.F. mm	8.9	10.0	1[illegible].6
	Weight kg	63	[illegible]4.3	83.3

subjects concerning other manifestations of high blood pressure the subjects in B P IV (S_3D_4, S_4D_3, S_4D_4) was divided into fat (≥18 4 mm subscapular skinfold) and non fat (<18 4 mm subscapular skinfold) using upper quartile value of subscapular skinfold as a dividing line (table 28) In all groups, the fat hypertensive subjects had a higher frequency of other manifestations of high blood pressure The results showed that the fat hypertensive subjects had as advanced or more advanced high blood pressure than the non fat hypertensive subjects

Prevalence of fat subjects

The number of fat subjects (subscapular skinfold thickness ≥18 4 mm) in S_1D_1 ($n=26$), S_2D_2 ($n=466$) and S_4D_4 ($n=32$) were 19 per cent, 22 per cent and 52 per cent, respectively This indicates that there were in this sample a considerable number of fat subjects with low blood pressure and intermediate blood pressure

Hypertensive eye-ground changes and subscapular skinfold thickness and weight

Hypertensive eye ground changes (focal narrowings and/or attenuated arterioles) were used as manifestations of high blood pressure independent of arm size The *total* series was divided into three groups using the 33 and 66 percentiles of subscapular skinfold thickness and weight as dividing lines The prevalence of eye ground changes in these groups are presented in table 29 and 30 There was a significant rise in rate of eye ground changes with increase in subscapular skinfold and weight

The total series was then divided into

TABLE 27 Comparison of subscapular skinfold thickness weight and height between subjects in different blood pressure groups

	S_1D_1 $n=26$	S_2D_2 $n=468$	S_4D_4 $n=33$
Subscapular skinfold (mm)	11 8	14 7[2]	19 9
S D	4 6	5 3	9 1
P[1]	<0 01		<0 001
Weight (kg)	68 4	75 2[2]	82 9[3]
S D	9 6	9 9	15 4
P[1]	<0 001		<0 001
Height (cm)	173 8	175 2[2]	175 2[3]
S D	7 4	6 0	6 5
P[1]	n s		n s

[1]) The differences S_1D_1–S_2D_2 and S_4D_4–S_2D_2 were tested

[2]) Data from two subjects were missing

[3]) Data from one subject was missing

TABLE 28 Comparison between fat hypertensive subjects and non fat hypertensive subjects in B P[1]) IV (S_3D_4, S_4D_3, S_4D_4) with reference to other manifestations of high blood pressure in per cent

	B P IV (S_3D_4, S_4D_3, S_4D_4)	
	Fat subjects S F ≥ 18 4 mm $n=28$	Non fat subjects S F < 18 4 mm $n=33$
Focal narrowings	61%	46%
Attenuating arterioles	57%	33%
Ecg findings (Minnesota Code III 1 IV 1-3 V 1-3)	29%	18%
LVH (x ray)	57%	33%

[1]) Subjects with antihypertensive treatment were excluded

nine groups using the 33 and 66 percentiles of subscapular skinfold thickness and weight as dividing lines The prevalence rate of eye ground changes are presented in table 29 In table 30 the mean value of systolic blood pressure, diastolic blood pressure subscapular skinfold and weight for the nine combinations are shown

In subjects with light weight (<70 8 kg) there was no increase in rate of eye ground changes with rise in skinfold thickness In lean subjects (subscapular skinfold <12 1 mm) there was no increase in prevalence of eyeground changes with rise in weight On the other hand in the heavy subjects (≥ 79 5 kg) there was an increase with rise in skinfold thickness ($\chi^2 = 6$ 52 1 D F $p<0$ 05) The fat subjects (subscapular skinfold ≥ 16 7 mm) showed also an increase in rate of eye ground changes with rise in weight ($\chi^2 = 7$ 09 1 D F <0 01) A modification of χ^2 test was

TABLE 29 Prevalence rate of hypertensive eye ground changes in different combinations of weight and subscapular skinfold thickness

Subscapular skinfold (mm)	Weight (kg) <33 percentile	>33 percentile–<66 percentile	≥66 percentile
>66 percentile	6 9% (n = [illegible]9)	8 3% (n = 72)	[illegible]0 5% (n = 182)
>33 percentile–≤66 percentile	[illegible] 6% (n = 72)	9 1% (n = 132)	13 9% (n = [illegible]9)
<33 percentile	0 0% (n = 182)	6 3% (n = [illegible]9)	0 0% (n = 2[illegible])

TABLE 30 Blood pressure weight and skinfold thickness in subjects in different combinations of weight and skinfold thickness

Subscapular skinfold		Weight <33 percentile	≥33 percentile–<66 percentile	>66 percentile
66 percentile	S B P mm Hg	135	136	147
	D B P mm Hg	8[illegible]	9[illegible]	9[illegible]
	S F mm	[illegible]0 6	19 3	23 [illegible]
	Weight kg	66	75 1	90 1
33 percentile <66 percentile	S B P mm Hg	135	140	14[illegible] 3
	D B P mm Hg	88	92	94 3
	S F mm	13 9	14 3	14 6
	Weight kg	66 4	[illegible]	84 6
<33 percentile	S B P mm Hg	130	135	131
	D B P mm Hg	86	90	88
	S F mm	8 9	10 0	10 6
	Weight kg	63 [illegible]	74 2	83 3

used (3) The four extreme groups were of particular interest Heavy subjects without fatness and fat subjects of light weight had roughly the same prevalence of eye ground changes as lean subjects of light weight The combination of heaviness and fatness gave a high rate of hypertensive eye ground changes

Discussion

Skinfold measurement, because of the rapidity and simplicity and the considerable evidence of the relationship to body fat composition, serves as a useful indication of fatness of individuals and groups (24) (45) (47) (55) (106) It is estimated that something like half of the total body fat is accounted for in the subcutaneous fat layer (106) For purposes of classifying individuals along the leaness fatness continuum in field surveys, the goal is to select a minimum number of sites suitable for skinfold measurements and provide, in proper combination, the optimal measure of relative adiposity The selection of sites involves several considerations such as accessibility, precision in locating the site, relative homogeneity of the layer of skin and subcutaneous fat in a given region and validity as an index of total fat Various workers have used a number of sites, including the triceps, subscapular abdominal, hip, pectoral and calf For the general population the Committee on Nutritional Anthropometry of the National Research Council in U S A (19) has recommended the triceps and the subscapular skinfolds as good indices of overall fatness The World Health Organization has in 1963 recommended measurement of skinfold thickness over the triceps and the subscapular areas (152) In the present study, measurement of subscapular skinfold was done, but no measurement of triceps skinfold thickness was done, and the former is therefore used in the analysis

Truedsson studied variation of arterial blood pressure with skinfold thickness in a series consisting of various categories of males and females who felt healthy and were in full time employment (160) In the males there was no correlation between skinfold thickness and blood pressure when the influence of age was eliminated while for females it remained significant The number of male subjects was 85 (age 10–69) and only two subjects had ≥175 mm Hg systolic blood pressure and one subject >100 mm Hg diastolic blood pressure

Palmai (123) made serial measurements of body weight skinfold thickness and arterial blood pressure over a period of eleven months from January to November in a closed male community (age 24–47) in a cold wet sub antarctic environment No significant alternation in body weight was demonstrated but a seasonal variation in skinfold thickness was observed The change in skinfold thickness was accompanied by a closely parallel change in systolic and diastolic blood pressure The range of blood pressure was not given but the mean values of the systolic and diastolic pressures were 124 6 and 75 0 mm Hg in January and 142 1 and 91 3 mm Hg in August There was a high correlation between individual values for skinfold thickness and blood pressure throughout the year The results support the view that blood pressure is closely related to obesity and that skinfold thickness is superior to indices based on body weight

Whyte studied 100 apparently healthy men 20 to 40 years of age and found *that there was a highly significant, positive relationship between skinfold thickness and blood pressure* (164) However when age height weight and size of arm were held constant the partial correlation coefficients became insignificant Lindegård found a positive correlation between blood pressure and skinfoldthickness in a group of men aged 20–30 (33)

The relationship between obesity and blood pressure have been investigated in a population study by Reid *et al* (139) They found that the partial correlations between subscapular skinfold thickness (weight constant) and blood pressure was significant ($r = 0.17$)

In the studies of Keys *et al* (85) it was clear that *in most of 17 population* studies there were significant increases of the prevalence of high blood pressure with increasing obesity *measured* by skinfold thickness The exception was a Serbian village Velika Krsna where high blood pressure showed little relationship to obesity Compared with the averages for all samples of men in these studies the men of Velika Krsna were relatively underweight and markedly thin

In the present study the high blood pressure group S_4D_4 had significantly higher subscapular skinfold and weight *Compared with the non fat hypertensive* subjects the fat hypertensive subjects had as many or more manifestations of high blood pressure These findings ruled out the assumption that the fat subject with *high blood* pressure *had* an innocent type of blood pressure elevation.

In the Goteborg sample of men some fractions of fat subjects had low blood pressure or intermediate blood pressure If Alexander s finding is correct (1), that fat subjects have elevated cardiac output, it is evident the computed vascular resistance is low in this group of men

In considering the relation between obesity and arterial pressure the influence of arm circumference on the measuring of the indirect blood pressure is of interest Ragan and Bordley (1941) found that the agreement between auscultatory and intraarterial measurement of systolic blood pressure was affected by the size of the subjects arm (137) In subjects with thin arms the auscultatory measurements were usually too low When the subjects *in their study* with *upper* arms *greater* than 35 cm or less than 24 cm in circumference were excluded, the auscultatory *systolic readings came with* ± 10 *mm* Hg of intra arterial systolic pressure in 83% of comparisons The auscultatory measurements of diastolic blood pressure were usually higher than the intra arterial measurements The mean deviation of auscultatory from the intra arterial measurements was 8 mm Hg The positive deviation became more pronounced when the arm size was more than 35 cm

Using Ragan and Bordley s data Pickering *et al* (132) worked out a table of corrections that could be applied to the cuff blood pressure in relation to arm *circumference* when a standard cuff for adults is used They pointed out that these corrections were worth making for large groups of individuals *The connection* between cuff blood *pressure* and arm circumference has obvious implications in population studies since arm circumference varies with sex age, weight and occupation.

In recent years several authors have reported comparisons between direct and indirect blood pressure Ries (1960) found that the blood pressure recorded by the auscultatory method probably does not deviate from that obtained by direct measurement, even in obese persons (142)

In a comprehensive study by Berliner it was clear that the discrepencies between the direct and indirect methods were usually minor among the lean and moderately obese subjects and could be in either direction (13)

Holland and Humerfelt (1964) made a comparative study of direct and indirect blood pressure measurements They could not confirm the work of Ragan and Bordley as they failed to find a relationship between arm circumference and the difference between intra arterial and cuff pressures (68)

Alexander (1961) found in grossly obese subjects that the blood pressure determined nearly simultaneously by direct intra arterial and indirect cuff methods, agreed to within 20 mm Hg systolic and 10 mm Hg diastolic blood pressure on about half the occasions The cuff method gave falsely low estimates as frequently as it did high (1)

Karlefors (80) found that there was a good agreement between the auscultatory and the directly recorded systolic blood pressure For the diastolic pressures a significant systematic difference was obtained with the directly recorded pressures on the average 11 3 mm Hg lower than the indirectly measured pressures The arm circumference of the subject had no detectable influence on the accuracy of the estimation of systolic pressure by the indirect method For the diastolic pressure there was an overestimation of 15 mm Hg in 9 subjects with thick arms compared to an overestimation of 7 5 mm in 9 subjects with thin arms Test of the significance of the difference was not done

In the literature from the recent years there seems to be agreement that the differences between intra arterial and cuff blood pressure are not generally affected by arm circumferences The relationship found between obesity and high blood pressure in our study cannot, therefore, be explained by this factor Any attempt to correct sphygmomanometer pressure readings for arm circumference will reduce the real influence of obesity

Eye ground changes are manifestations of hypertensive disease which are independent of arm size The finding that the prevalence of subjects with eye ground changes increased about three times with increase in subscapular skinfold and weight strongly support the assumption that the relationship between obesity and high blood pressure is a genuine one Of interest was the observation that the highest prevalence of eye ground changes was not found in the mere fat and the mere heavy subject but in the subject who was both fat and heavy It is difficult to explain this finding A hypothesis is that when a subject with high amount of other body components for example muscle, adds to that fatness he develops high blood pressure more easily

Few have discussed the explanation for higher blood pressure in fat subjects Whyte (164) believed that increased body size demanded an increased cardiac output and that this was forced into a vascular system that had not increased enough in

volume Another possibility according to Whyte was that external body features might reflect quantitative differences in electrolyte or renin angiotensin metabolism

It cannot be assumed that because subjects with high blood pressure often are fat that it is the fatness which causes the high blood pressure since both events may be the result of a third set of antecedent events for example physical inactivity There is some support for such an hypothesis in the literature From the Framingham Study (36) it was apparent that there were no significant differences in daily intake of calories protein fat and cholesterol when the study subjects were classified into four blood pressure groups The relationship between high blood pressure and physical activity has not been studied in detail but Morris and Crawford in a necropsy survey found that subjects with light work load had evidence of high blood pressure more often than workers with heavy work load (110 b)

On the basis of the results of these investigations, it can be concluded that fatness seems to be of importance for the variation of blood pressure The reason for this is not clear and further investigations seem necessary to clarify more precisely the underlying mechanisms A follow up study of subjects in whom the fat factor and muscle factor have been adequately studied should be of great interest The problem is that accurate methods (body density, total body water whole body counter) are not yet available for population studies

Obesity has an important influence on several factors studied here—heart volume ECG biochemical variables and social factors In further analysis of the results the relationship between obesity and high blood pressure must be taken into consideration (Appendix)

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Eye ground changes are manifestations of hypertensive disease which are independent of arm size. The finding that the prevalence of subjects with eye ground changes increased about three times with increase in subscapular skinfold and weight strongly support the assumption that the relationship between obesity and high blood pressure is a genuine one. Of interest was the observation that the highest prevalence of eye ground changes was not found in the mere fat and the mere heavy subject but in the subject who was both fat and heavy. It is difficult to explain this finding. A hypothesis is that when a subject with high amount of other body components for example muscle adds to that fatness he develops high blood pressure more easily.

Few have discussed the explanation for higher blood pressure in fat subjects. Whyte (164) believed that increased body size demanded an increased cardiac output and that this was forced into a vascular system that had not increased enough in

Table 32 shows the differences in the mean values of plasma creatinine between S_1D_1 S_4D_4 and treated subjects when compared to controls from S_2D_2 matched for height weight and subscapular skin fold (Appendix) There were no significant differences among the groups

Table 33 shows the differences in mean value of morning urine osmolality between S_1D_1 S_4D_4 and treated subjects when compared to matched controls Morning urine osmolality was significant lower in S_4D_4 and treated subjects

Renal disease

The most common disease affecting the kidney and urinary tract in this population of men was urinary calculus In the total series 58 subjects (6 8%) had a history of urinary calculus When the subjects were examined in 1963 proteinuria occured in 20 cases (2 3%)

Table 34 presents the frequency of these two phenomena in B P I IV There was a positive and significant relationship between increase in blood pressure and frequency of urinary calculus ($\chi^2=34$ 1 1 D F $p<0$ 001) and proteinuria (χ^2—7 47 1 D F $p<0$ 01)

The number of subjects with history of other renal diseases is rather low (21/800) In the groups S_4D_4 and treated subjects there is just one subject which had such a diagnosis—a man with renal tuberculosis

Discussion

An association between renal disease and high blood pressure has long been

TABLE 3 Difference in mean value of plasma creatinine between S D S_4D_4 treated subjects and their controls matched for height weight and subscapular skinfold thickness

	Difference (mg/100 ml)	S	n	Significance of the difference from control group
S D Matched controls	+0 0	0 [illegible]1	[illegible]6	n.s
S D_4 Matched controls	+0 03	0 22	3[illegible])	n s
Treated subjects Matched controls	+0 [illegible]1	0 1	14	n s

) Data from one subject was missing

TABLE 33 Difference in mean value of morning urine osmolality between S_1D S_4D_4 and treated subjects and their controls matched for height weight and subscapular skinfold thickness.

	Difference (mOsmol/kg H_2O)	S	n	Significance for difference
S D Matched controls	+ 3	[illegible]	[illegible]4[2])	n.s.
S D_4 Matched controls	−117	[illegible]15	3)	$p<0$ 01
Treated subjects Matched controls	−1 6	[illegible]5	14	$p<0$ 05

) Data from one subject was missing

[2]) Data from two subjects were missing

The problem of high blood pressure as a secondary phenomenon of renal disease is one of the current controversies Wilson, in the Prague Symposium (1961), listed 12 actual problems in high blood pressure research (166) Item 2 was "In view of the more exact methods of diagnosis now available, how large a proportion of cases, masquerading under the diagnosis of essential hypertension, are really due to some local disease—e g of the kidneys or endocrine glands?" This chapter is an attempt to elucidate the relationship between some renal function test, renal disease and blood pressure

Methods

The study included the collection of records from previous hospital visits The subjects were also asked about history of urinary calculus It was possible to verify a history of urinary calculus by x ray in 35 subjects In 23 subjects there was a history of stone release The group of subjects with urinary calculus consisted therefore of 58 subjects The renal function tests used in this study were plasma creatinine and morning urine osmolality

Results

Renal function

The mean values and standard deviations of renal function tests in different blood pressure groups are presented in table 31

TABLE 31 Mean value of plasma creatinine and morning urine osmolality in S_1D_1 S_2D_2 S_4D_4 and treated subjects

	S_1D_1	S_2D_2	S_4D_4	Treated subjects
Plasma creatinine (mg/100 ml)				
Mean	1 01	1 03	1 05	1 24
S	0 12	0 17	0 20	0 [illegible]0
n	26	467[1]	33	14
Morning urine osmolality (mOsmol/kg H_2O)				
Mean	885	868	749	735
S	155	169	145	166
n	24[2]	46[3]	32[4]	14

[1]) Data from one subject was missing

[2]) Data from two subjects were missing

[3]) Data from six subjects were missing

[4]) Data from one subject was missing

patients with proteinuria indicate that proteinuria signifies chronic renal disease (86) Of 51 asymptomatic patients with mild intermittent proteinuria and normal renal function 61% had specific anatomical lesions of the glomeruli and another 8% had nonspecific glomerular abnormalities (114)

In principle this result agrees with those of McDonough *et al* who in Evans County found a higher prevalence of proteinuria at higher levels of blood pressure (99)

The difference in morning urine osmolality between S_1D_1 S_4D_4 and treated subjects was also investigated S_4D_4 and treated subjects had significantly lower urine osmolality The possible reason for this reduced osmolality may be renal involvement with reduced concentration capacity and/or altered rhythm of water and solute excretion

The relationship between the concentration capacity and elevated blood pressure in population studies has not previously been investigated Corcoran and Page found in a series of patients suffering from high blood pressure without known cause that urea clearance tended to remain within normal limits while concentrating power was usually impaired (31) A defect in the concentrating mechanism early in the course of essential hypertension has been reported by some authors (10) (37) (149) According to other authors (69) the ability to elaborate a concentrated or dilute urine does not become impaired during the course of high blood pressure of unknown cause except in those few patients who enter the accelerated malignant phase Morning urine osmolality without instructions about fluid reduction the evening before cannot be regarded as maximal urine osmolality The results in the present study are therefore difficult to explain They may indicate a defect in the concentrating power of the kidney in subjects of S_4D_4 and the treated subjects Another explanation is an increased drinking during the night in the two elevated blood pressure groups but there is no support in the literature for a change in drinking habits in high blood pressure A third possibility is an altered rhythm of water and solute excretion but there are no data in the present study which can elucidate such a suggestion

According to American Heart Associations Committee on Criteria for Diagnosis of Disease high blood pressure of known cause is rare and it would seem unnecessary in epidemiological studies to make special efforts to identify such cases (42) This view has been confirmed by two groups (111) (120) one of which reported one and the other two secondary cases of high blood pressure In the present study there was one subject with a history of renal disease in S_4D_4 and treated subjects ($n=47$)

Of interest in this connection is the result of Fodor and Hejl (56) In a study of a random sample of males aged 60–64 years taken from the general population of Prague a subsample of 41 subjects with high diastolic blood pressure was hospitalized for a week and examined with elaborate renal function tests The authors concluded that in this age group the ratio of renal artery stenosis and renal hypertension generally was of negligible significance

Knowledge of the relationship between urinary calculi and elevated blood pres

recognized Elevated blood pressure often occurs in acute glomerulo nephritis and almost invariably in the advanced stages of the disease Likewise, high blood pressure is commonly associated with chronic pyelonephritis, diabetic nephropathy, polycystic renal disease and the renal disease which occurs in systemic lupus erythematosus and polyarteritis nodosa High blood pressure may also be observed when the renal artery is stenosed On the other hand renal vasoconstriction, structural arteriolar changes and parenchymal damage, all may be found in the kidneys of patients with high blood pressure of unknown cause This association has suggested the possibility that high blood pressure of unknown cause may also be renal in origin In spite of intensive investigations of the anatomy and physiology of the kidney, it is still not possible to say whether the kidney is responsible for blood pressure elevation or whether the renal structural and functional abnormalities found in high blood pressure are manifestations of an extrarenal factor which affects the kidney secondarily

The problem of renal function in population studies of elevated blood pressure has not been subjected to adequate investigation One of the reasons for this is probably that most measures of glomerular and tubular function require a degree of subject co operation and manipulation unobtainable in field studies High blood pressure of unknown cause is diagnosed by a process of exclusion which involves an intensive clinical study of each individual Miall has pointed out the fact that this approach is so difficult to apply in a epidemiological study that no attempt, so far as he knows, has yet been made to do so (108) On the other hand several aspects of renal function can be assessed in populations by standard clinical methods In this study tests for urine protein, serum creatinine and osmolality of morning urine have been used There was no significant difference in serum creatinine between S_1D_1, S_4D_4 and treated subjects and matched controls This was expected, as a substantial rise in serum concentration of creatinine is found only in severe reduction in glomerular filtration rate

There was a positive and significant trend in frequency of proteinuria with increase in blood pressure Proteinuria is almost always present when there is a disease of the renal parenchyma and it is not a feature of high blood pressure of unknown cause Follow up studies on

TABLE 34 Prevalence rate of urinary calculus and proteinuria in B P I–IV

	B P I (S_1D_1 S_1D_2 S_2D_1) ($n=89$)	B P II (S_2D_2) ($n=468$)	B P III (S_2D_3 S_3D_1 S_3D_3) ($n=$ [illegible])	B P IV (S_3D_4 S_4D_3 S_4D_4 and treated subjects) ($n=$ [illegible])
Urinary calculus[1])	1 1%	6 6%	7 3%	13 3%
Proteinuria[2])	1 1%	1 4%	3 2%	6 [illegible]%

[1]) $\chi^2=34$ 1 D F $p<0$ 001

[2]) $\chi^2=7$ 4 1 D F $p<0$ 01

patients with proteinuria indicate that proteinuria signifies chronic renal disease (86) Of 51 asymptomatic patients with mild intermittent proteinuria and normal renal function 61% had specific anatomical lesions of the glomeruli and another 8% had nonspecific glomerular abnormalities (114)

In principle this result agrees with those of McDonough *et al* who in Evans County found a higher prevalence of proteinuria at higher levels of blood pressure (99)

The difference in morning urine osmolality between S_1D_1 S_4D_4 and treated subjects was also investigated S_4D_4 and treated subjects had significantly lower urine osmolality The possible reason for this reduced osmolality may be renal involvement with reduced concentration capacity and/or altered rhythm of water and solute excretion

The relationship between the concentration capacity and elevated blood pressure in population studies has not previously been investigated Corcoran and Page found in a series of patients suffering from high blood pressure without known cause that urea clearance tended to remain within normal limits while concentrating power was usually impaired (31) A defect in the concentrating mechanism early in the course of essential hypertension has been reported by some authors (10) (37) (149) According to other authors (69) the ability to elaborate a concentrated or dilute urine does not become impaired during the course of high blood pressure of unknown cause except in those few patients who enter the accelerated malignant phase Morning urine osmolality without instructions about fluid reduction the evening before cannot be regarded as maximal urine osmolality The results in the present study are therefore difficult to explain They may indicate a defect in the concentrating power of the kidney in subjects of S_4D_4 and the treated subjects Another explanation is an increased drinking during the night in the two elevated blood pressure groups but there is no support in the literature for a change in drinking habits in high blood pressure A third possibility is an altered rhythm of water and solute excretion but there are no data in the present study which can elucidate such a suggestion

According to American Heart Associations Committee on Criteria for Diagnosis of Disease high blood pressure of known cause is rare and it would seem unnecessary in epidemiological studies to make special efforts to identify such cases (42) This view has been confirmed by two groups (111) (120), one of which reported one and the other two secondary cases of high blood pressure In the present study there was one subject with a history of renal disease in S_4D_4 and treated subjects ($n=47$)

Of interest in this connection is the result of Fodor and Hejl (56) In a study of a random sample of males aged 60–64 years taken from the general population of Prague a subsample of 41 subjects with high diastolic blood pressure was hospitalized for a week and examined with elaborate renal function tests The authors concluded that *in this* age group the ratio of renal artery stenosis and renal hypertension generally was of negligible significance

Knowledge of the relationship between urinary calculi and elevated blood pres-

sure has previously been insufficient The present investigation shows a positive relation between frequency of calculi and rise in blood pressure The primary cause of urinary stone formation is unknown For further studies on the problem of urinary calculi and blood pressure it seems necessary to do a follow up study of normotensive subjects with urinary calculi in order to gain better insight as to cause and effect

The relationship between renal disease and blood pressure is complicated by the fact that it is difficult to restrict the entity under study The number of subjects with a history of kidney disease is small in the highest blood pressure group In S_4D_4 and among treated subjects there was one man (1/47) On the other hand, the number of subjects with pathological findings from kidney and urinary tract is large 29 subjects of S_4D_4 and treated subjects ($n=47$) had one or more of the following proteinuria, low urine osmolality (<780 mOsmol/kg H_2O) and urinary calculus, compared to 12 subjects in the matched controls ($\chi^2=11\ 96$ $p<0\ 001$) This discrepancy can be explained in several ways There is a large group of subjects with undetected renal diseases such as glomerulonephritis, chronic pyelonephritis, diabetic glomerulosclerosis, stenosis of the renal artery and polycystic kidney disease, with high blood pressure as the first symptom In a random sample of men aged 50 this explanation seems to be less probable, but it is difficult to exclude the possibility in a population study The other explanation is that high blood pressure is the cause of the renal damage However, many other relationships may exist and further studies are required before a complete answer can be given to the question as to how renal disease and blood pressure are related

In the analysis of the data from this study it was clear that the low blood pressure groups contained an increased amount of subjects with a history of lung diseases. This finding is presented in this chapter. In order to assess the influence of current infectious disease on blood pressure, the sedimentation rate was used as a rough index of infectious disease.

The diagnosis of pulmonary tuberculosis and pneumonia were taken from the hospital records. As has been found in the present study S_1D_1 and S_4D_4 showed significant differences in weight and subscapular skinfold thickness as compared to S_2D_2. In order to take these findings into consideration S_1D_1 and S_4D_4 were compared with controls matched for height, weight and subscapular skinfold from S_2D_2. The procedure is described in Appendix (p. 74).

Results

Lung disease

Table 35 shows the prevalence rate of subjects with the hospital diagnosis of pneumonia and pulmonary tuberculosis in B.P. I-IV. There was a significant decrease in subjects with pneumonia and pulmonary tuberculosis with rise in blood pressure ($\chi^2=12.03$, 1 D.F., $p<0.001$ and $\chi^2=4.69$, 1 D.F., $p<0.05$).

TABLE 35. Prevalence rate of subjects with hospital diagnoses of pneumonia and pulmonary tuberculosis in B.P. I-IV

	B.P. I (S_1D_1, S_1D_2, S_2D_1) ($n=89$)	B.P. II (S_2D_2) ($n=468$)	B.P. III (S_2D_3, S_3D_2, S_3D_3) ($n=2$[illegible]0)	B.P. IV (S_3D_4, S_4D_3, S_4D_4 and treated subjects) ($n=75$)
Pneumonia[1]	13.5%	[illegible].2%	3.5%	1.3%
Tuberculosis of the pulm.[2]	10.1%	4.8%	3.6%	2.[illegible]%

[1] χ^2 12.03 $p<0.001$

[2] χ^2 4.69 $p<0.05$

TABLE 36. Mean value of sedimentation rate in S_1D_1, S_2D_2, S_4D_4 and treated subjects.

	S_1D_1	S_2D_2	S_4D_4	Treated subjects
Sedimentation rate				
Mean	10.7	7.7	8.4	8.4
S	8.[illegible]	7.5	7.[illegible]	7.0
n	26	463[1]	33	14

[1] Data from five subjects were missing

Sedimentation rate

As a rough index of infectious disease the sedimentation rate was used. Table 36 shows the mean value for sedimentation rate in S_1D_1, S_2D_2, S_4D_4 and treated subjects. The differences in sedimentation rate between S_1D_1, S_4D_4 and treated subjects and their controls matched for height, weight and subscapular skinfold thickness are presented in table 37. The sedimentation rate was significantly higher in S_1D_1 as compared to the control group.

Discussion

Low blood pressure has not interested the epidemiologist much. Clinicians have considered it of importance as an explanation for vague complaints of fatigue and circulatory inadequacy. However, subjects with low blood pressure are worth a more careful study. They have a higher life expectancy (17b) and a relative freedom from arteriosclerotic cardiovascular complications (77). Little is known which can explain this interesting finding. The low blood pressure in "primitive" populations has been explained by undernutrition and infectious diseases. An interesting question is if subjects from an urbanized area with low blood pressure suffer of malnutrition or infectious disease more often than those with normal or high blood pressure.

On the basis of the results obtained in the present study it can be assumed that there is a relationship between a history of infectious lung diseases (pneumonia and tuberculosis of the lung) and low blood pressure. Of interest is a finding from this group of men that also some signs in the x ray films of the lungs taken when the subjects were examined in 1963, were related to blood pressure. Thus the prevalence rate of calcifications in the hilar nodes and depressed diaphragms decreased with increase in blood pressure ($\chi^2 = 9.27$ 1 D.F. $p < 0.01$ and $\chi^2 = 12.69$ 1 D.F. $p < 0.001$). Detailed results will be reported separately (159). The increased sedimentation rate in S_1D_1 supports the assumption that infectious disease can be involved in the regulation of the blood pressure of these men. Attention has already been directed to the relation between skinfold thickness and blood pressure. Of interest is the finding that S_1D_1 had significantly lower skinfold as compared to S_2D_2.

Brozek and Keys found (23) when they studied the effect of drastic food restriction on cardiovascular dynamics of 36 normotensive young subjects, that food restriction usually lowered blood pressure. The mean systolic blood pressure for the

TABLE 37. Differences in sedimentation rate between S_1D_1, S_4D_4 and treated subjects and their controls matched for height, weight and subscapular skinfold thickness

	Difference (mm/l hour)	S	n	Significance of the difference
S_1D_1 – Matched controls	+5.0	10.6	26	$p < 0.05$
S_4D_4 – Matched controls	+1.0	6.2	30[1])	n.s.
Treated subjects – Matched controls	+1.4	9.2	14	n.s.

[1]) Data from three subjects were missing

entire group fell from 105 5 mm to 94 7 of mercury a decrease of 11 1 per cent

During the field work in Finland, Karvonen mentioned that the examiners gathered the impression that men with chronic bronchopulmonary diseases rarely had high blood pressure (82) However when blood pressure reading were related to the results of F E V and Peak flow tests no correlation was observed

In a random sample of 238 New Guinea males, those with large spleens (resulting from chronic malaria) and low peak expiratory flow rates (regarded as the result of respiratory infection) had lower blood pressure than subjects with small spleens and high peak flow rates (102)

Experimental and epidemiological observations from this study and others indicate that infectious disease and the nutritional status are of importance for the height of the blood pressure

When the blood pressure of different ethnic groups are to be compared information on effects of infectious diseases and undernutrition is of interest In order to establish comparability with these men aged 50 some information about their nutritional and infectious status is given in table 38 The mean value standard deviation and 25th, 50th and 75th percentiles, are given for subscapular skinfold thickness, weight height total serum protein, hematocrit and sedimentation rate

In this study only renal and infectious diseases have *been investigated in relationship* to blood pressure However, major interest in disease associated with high blood pressure has been focused on cardiovascular disease and particularly, coronary heart disease and cerebrovascular disease In this investigation it was difficult to study this relationship because of lack of subjects with these diseases There were only eight subjects with myocardial infarction treated in hospital and two subjects with cerebrovascular disease There was no clear trend concerning blood pressure among these few cases

TABLE 38 Nutritional and infectious status of the study population

	n[1])	Mean	S D	Percentile		
				25th	50th	75th
Nutritional factors						
Subscapular skinfold thickness (mm)	8[illegible]1	1[illegible] 8	6 [illegible]	10 6	14 1	18 4
Weight (kg)	851	[illegible]5 9	11 0	68 0	74 5	82 9
Height (cm)	8[illegible]	1[illegible]5 0	6 0	1[illegible]0 [illegible]	174 8	180 6
Total serum protein (g per 100 ml)	853	7 12	0 44	68 4	[illegible] 01	[illegible] 5
Hematocrit (%)	8[illegible]	44 9	3 3	42	4[illegible] 9	48 [illegible]
Infectious factors						
Sedimentation rate (mm 1 hour)	849	7 8	7 4	3 1	5 1	10 6

[1]) The sample examined consisted of 8[illegible] subjects. The difference in number of subjects was caused by missing data.

HEART VOLUME

It is generally agreed that the heart responds to an increase in systemic arterial pressure with left ventricular hypertrophy It is also known that there is a strong relationship between heart weight and systolic blood pressure in patients dying of high blood pressure (53) A correlation has been found between heart weight and the severity of hypertensive disease in experimental hypertension in rabbit (128) The syndrome of congestive failure with cardiac dilatation has been one of the major causes of death in patients with high blood pressure All these factors have certainly contributed to the general belief that high blood pressure gives rise to enlargement of the heart in the living subject Humerfelt, however, in his extensive review of the literature on heart volume found few studies on the influence of blood pressure upon heart volume (70) Josephsen did not find any demonstrable correlation between the duration or severity of hypertension and the size of the heart on x ray in a series of 80 hypertensive hospitalized patients (75) Amundsen (2) reported, that in hospital subjects with high blood pressure (160/90 or more) without other evidence of cardiac or renal disease only in the group of males aged 40–59 was the heart volume significantly increased On the other hand Bechgaard found that the number of patients with radiological evidence of increased cardiac size rose with increase in systolic blood pressure (11)

Humerfelt (70) was the first to show, in a representative population study, that the heart volume index (heart volume per *sq m* body surface area) increases almost linearly with age in women in all blood pressure groups In men, the mean values describe a slightly S shaped curve There was a slight but consistent elevation of this curve with higher blood pressure The statistical analysis showed that the heart volume index increased in men by 9 ml for each 15 mm Hg diastolic blood pressure

From Humerfelt s study there is no doubt that age has an important influence upon heart volume There also seems to be a relationship between an increase in the heart size and a rise in blood pressure How much of the cardiac enlargement depends upon aging is still unsettled In Humerfelt's study, using groups with an age range of 10 years the slight increase in heart volume with rising blood pressure may depend upon the fact that the age was not adequately standardized

Methods and material

The method used for calculating the heart volume is presented on page 21

When x ray determination of cardiac size is used it must be realized that there are technical difficulties in exact measurement It is not possible by any method to embrace all bodily and circulatory factors which influence heart size The most important factor is undoubtedly body build It was clear to Harvey (66)

that the mass of the heart depends upon *body build and more particularly* upon the state of body musculature In heart volume studies, the most common method is to relate the absolute volume to body surface area or to weight The correlation coefficient for either is of the same order (for men between 0 48–0 [illegible]) *(104)* In this study the heart volume is related to body surface area

In order to reduce as much as possible the influence of body build upon heart volume controls for all those subjects in groups S_1D_1 S_4D_4 and treated subjects were selected and matched according to height weight and skinfold thickness (appendix p 74)

Results

Heart volume

Table 39 shows the mean value for total and relative heart volume (ml/m^2 BSA) in prone and standing position in S_1D_1 S_2D_2 S_4D_4 and treated subjects There were significant differences between S_4D_4 and S_2D_2 in relative heart volume both in prone and standing position (significance for differences $p<0\ 01$ and $p<0\ 001$) There was no significant difference between S_1D_1 and S_2D_2 in these two variables

Tables 40 and 41 show that when height, weight and skinfold thickness were taken

TABLE 39 Mean value of total heart volume in prone and standing position and relative heart volume (ml/m^2 BSA) in prone and standing position in S_1D_1 S_2D_2 S_4D_4 and treated subjects [1])

	S_1D_1	S_2D_2	S_4D_4	Treated subjects
Total heart volume				
Prone position				
mean	745	811	917	863
S	114	144	163	181
n	26	438	28	12
Standing position				
mean	672	743	823	799
S	112	129	126	117
n	26	460	3[illegible]	14
Relative heart volume (ml/m^2 BSA)				
Prone position				
mean	410	4[illegible]5	465	454
S	53	61	70	71
n	26	438	28	12
Standing position				
mean	369	390	416	417
S	53	[illegible]8	60	[illegible]4
n	26	459	32	14

[1]) The number in S_1D_1 S_2D_2 S_4D_4 and treated subjects were 26 468 33 and 14 respectively The difference in number of subjects was caused by missing data.

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Heart volume

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Tables 40 and 41 show that when height weight and skinfold thickness were taken

TABLE 39 Mean value of total heart volume in prone and standing position and relative heart volume (ml m² BSA) in prone and standing position in S D S_2D_2 S_4D_4 and treated subjects [1])

	S D	S_2D_2	S_4D_4	Treated subjects
Total heart volume				
Prone position				
mean	45	811	917	863
S	114	144	163	181
n	°6	438	°8	12
Standing position				
mean	6 °	43	8°3	99
S	11°	1°9	126	117
n	°6	460	3	14
Relative heart volume (ml m² BSA)				
Prone position				
mean	410	425	465	454
S	53	61	0	1
n	°6	438	°8	12
Standing position				
mean	369	390	416	417
S	53	58	60	54
n	°6	459	3	14

[1]) The number in S_1D S_2D_2 S_4D_4 and treated subjects were °6 468 33 and 14 respectively The difference in number of subjects was caused by missing data

TABLE 40 Differences in total heart volume in prone position between S_1D_1, S_4D_4 and treated subjects and their controls matched for height, weight and subscapular skinfold thickness.

	Difference (ml)	S	n	Significance of the difference
S_1D_1—Matched controls	−29	130	6	n s
S_4D_4—Matched controls	+31	01	8	n s
Treated subjects—Matched controls	+63	14[illegible]	1	n s

into consideration there were no significant differences between the total heart volume in lying and standing position between S_1D_1, S_4D_4 *and treated* subjects and their matched controls

Discussion

Autopsy measurements of the heart are not comparable with proportions disclosed by x ray Cardiac size and shape change considerably after death owing to rigor mortis which empties most of the contents of the left ventricle and some of the right While cadaveric heart volume is determined largely by the status of its muscle the living heart size is decidedly influenced by its blood content which in turn is subject to great variations depending upon the status of the myocardium the circulating blood volume the venous return heart rate and the resistance against which the heart works

In determinations of cardiac size every effort should be made to exclude as far as possible all irrelevant intra and extra cardiac factors which affect cardiac filling Cardiac measurements should therefore be made at the same time of day preferably when fasting

The cardiac size and shape are profoundly influenced by the posture of the subject When one rises from the horizontal to the erect position the diaphragm descends and the heart resting on it follows the downward movement As a rule cardiac surface area is smaller and also the depth dimension is reduced Since many factors participate in the orthostatic reduction of cardiac size it is understandable that the amount of reduction may vary greatly Thus Zdansky (168) found that the cardiac volume determined by x ray occasionally was 200 cc less in the erect position than

TABLE 41 Differences in total heart volume in standing position between S_1D_1, S_4D_4 and treated subjects and their controls matched for height, weight and subscapular skinfold thickness

	Difference (ml)	S	n	Significance of the difference
S_1D_1—Matched controls	−3	141	6	n s
S_4D_4—Matched controls	+[illegible]	1[illegible]4	3	n s
Treated subjects—Matched controls	+80	184	14	n s

) Data from one subject was missing

when recumbent in individuals with no circulatory abnormality Consequently it is necessary to stabilize these factors as much as possible This goal is best attained with measurement in the recumbent position Heart volume measured by the method of Larsson and Kjellberg (91) in the prone position has been used frequently in Scandinavia since 1949 and has been used in the clinical routine in the Roentgenological department I of Sahlgrenska sjukhuset since 1957

Relative heart volume was greater in S_4D_4 as compared to S_2D The difference was rather small but significant 40 ml in prone and 27 ml in standing position For standing heart volume it is of the same order as that which Humerfelt found (70) On the other hand there was no significant difference in total heart volume when S_4D_4 was compared with a matched control group In other words when there were no differences in height weight and skinfold thickness between the groups there was no difference in heart volume in the standing and in the prone position Maurea Nylin and Sollberger (104) have discussed the problem using a quotient such as relative heart volume They pointed out that a quotient is constant only if the regression line between the components passes through the origin Since this seldom occurs most biological quotients are still correlated to their components thus creating what they called a tendential error They also showed that relative heart volume was correlated to the body surface area The quotient heart volume/weight was also correlated to body weight and the 'tendential error' was higher for the latter quotient

It is apparent from the present study that the number of subjects with signs of LVH (x ray) was greatest in S_4D_4 In the present series of men aged 50 the aortic width was measured on frontal film of the heart (159) When aortic width in S_4D_4 was compared with those of matched controls the mean value for S_4D_4 was found to be significantly higher Gustafsson and Friedenberg (63) in a comparison of different heart disease groups found the single most significant finding in patients with hypertensive cardiovascular disease was the marked prominence of the aortic knob Miall *et al* (151) found in a chest x ray survey of a rural population in Jamaica a high prevalence of subjects with aortic dilatation and among them a large fraction with blood pressure elevation These results are in agreement with Dotter and Steinbergs angiocardiographic finding (41) that aortic volume is increased in subjects with high blood pressure due to both elongation and dilatation This suggests according to Kreis (57) that the loss of distensibility of the aorta is a secondary rather than a primary effect Long continued elevation of pressure within large arteries results in stretching and a consequent loss of elasticity

The heart in subjects with high blood pressure has to overcome the increased peripheral resistance and the process has involved myocardial hypertrophy and elongation of the aorta These changes seem not to interfere with the size of the heart

SERUM—URIC—ACID

An increased prevalence of hyperuricaemia in patients with high blood pressure of unknown cause has been cited in several studies (21), (39) (87) (90)

The relationship between high blood pressure and uric acid has not been published in population studies and the first question to be answered is Has a representative sample of the hypertensive subjects in a population an elevated serum uric acid? In order to standardize for height, weight and subscapular skinfold thickness S_1D_1 and S_4D_4 were compared with matched controls from S_2D_2 (Appendix)

Results

The mean value of uric acid in S_1D_1, S_2D_2, S_4D_4 and treated subjects are presented in Table 42 In Table 43 the differences in mean value of uric acid between S_1D_1, S_4D_4, treated subjects and their matched controls are presented The only significant difference was found between S_1D_1 and its matched controls Subjects in S_1D_1 had lower serum uric acid

Discussion

Duncan and Dixon, 1960, described a family with a unique pedigree (43) The father and six of the seven siblings had

TABLE 42 Comparison of mean value of cholesterol (mg/100 ml) log 100 triglycerides (mMol/l) uric acid (mg/100 ml) and fasting blood sugar (mg/100 ml) in S_1D_1 S_2D_2 [1]) S_4D_4 and treated subjects

	S_1D_1	S_2D_2	S_4D_4	Treated subjects
Cholesterol (mg/100 ml)				
mean	242	246	251	265
S	47	41	36	57
n	26	468	33	14
log 100 triglycerides (mMol/l)				
mean	1 96	2 03	2 08	2 15
S	0 19	0 19	0 22	0 34
n	26	468	33	14
Uric acid (mg/100 ml)				
mean	4 52	5 22	5 32	6 59
S	0 74	1 04	0 95	1 66
n	25	462	33	13
Fasting blood sugar (mg/100 ml)				
mean	77	82	85	85
S	10	16	19	12
n	26	468	33	14

[1]) The numbers in S_1D_1 S_2D_2 S^4D^4 and treated subjects were 26 468 33 and 14 respectively The difference in number of subjects was caused by missing data.

TABLE 43 Difference in mean value of uric acid (mg/100 ml) between S_1D_1 S_4D_4 treated subjects and their controls matched for height weight and subscapular skinfold thickness

	Difference	S	n	Significance of the difference
S_1D_1 – Matched controls	−0 56	1 12	22[1])	$p<0$ 05
S_4D_4 – Matched controls	−0 35	1 3[illegible]	29[2])	n s
Treated subjects – Matched controls	+1 09	2 24	14	n s

[1]) Data from four subjects were missing

[2]) Data from four subjects were missing

hyperuricaemia while the mother and all the siblings had a raised blood pressure This finding raised the question whether a high serum uric-acid was common in patients with high blood pressure Dollery *et al* reported in the same year that in patients treated with penyridine mecamylamine or chlorothiazid 50 per cent of the males had a serum uric acid over 6 mg/100 ml (39)

Hyperuricaemia without typical gouty arthritis has been reported in hypertensives patients by several authors (21) (39) (72) (90) In an extensive study of 333 patients none of whom were taking antihypertensive drugs Breckenridge found that 27 *per cent* had elevated serum uric acid (21) According to Popert and Hewitt s (135) results this is five times the expected incidence in a general population of the same age

The association of high blood pressure with hyperuricaemia has been explained in three ways (21)

1) High blood pressure may arise as a result of hyperuricaemia

2) High blood pressure may cause hyperuricaemia

3) There is one aetiological factor common to these two conditions

Breckenridge discusses the first two explanations and favours the view that high blood pressure may cause hyperuricaemia by an abnormal handling of uric acid in the renal tubules system

The present series was analysed in order to see whether there was some common factor which could explain the relationship between high blood pressure and hyperuricaemia Brocks and Muller (22) found for example a significant correlation between obesity and uric acid ($r=0$ 26) Kolbel *et al* (90) reported in their study, in which a high proportion of hypertensive subjects had hyperuricaemia that the majority of the patients was fat Dunn *et al* found also an association between serum uric acid and obesity (44)

Uric acid was not significantly higher in S_4D_4 when this group was compared to matched controls The association of high blood pressure with hyperuricaemia found in hospital series therefore, could not be confirmed in this study This finding excludes the possibility that high blood pressure may arise as a result of hyperuricaemia It is also clear that obesity is not an aetiological factor common to these two conditions Hyperuricaemia in subjects with high blood pressure seems to be best explained as a phenomenon secondary to an advanced hypertensive disease

There is a close relationship between high blood pressure and vascular diseases. Myocardial infarction is one of the most common causes of death in hypertensive subjects. Atherosclerosis of the coronary arteries is the main etiological factor in myocardial infarction. It is a common belief that changes in lipid metabolism promote the development of atheroma.

Is the raised blood pressure the only factor which causes atheromatous vascular changes in hypertensive subjects or are lipid changes also present? In the present study blood lipids were studied in relation to blood pressure and in addition height, weight and skinfold thickness were taken into consideration (Appendix).

Results

A comparison of mean values of cholesterol and triglycerides between S_1D_1, S_2D_2, S_4D_4 and treated subjects is presented in table 42. The distribution of the triglycerides was skewed to the right and because of that the logarithmic value of original figures has been used. The differences in mean value of cholesterol between S_1D_1, S_4D_4, treated subjects and their controls matched for height, weight and subscapular skinfold are presented in table 44. There were no significant differences between the groups. Table 45 shows the differences in triglycerides between S_1D_1, S_4D_4 and treated subjects and their matched controls. There was a small but significant difference between S_4D_4 and the control group. No significant difference was found between S_1D_1 and treated subjects and their control groups.

Discussion

In the present series there were significantly higher triglycerides in subjects in S_4D_4 as compared to matched subjects. Cholesterol was not higher in S_4D_4 as compared to matched subjects. Previous investigations of the relationship between

TABLE 44. Differences in mean value of cholesterol (mg/100 ml) between S_1D_1, S_4D_4, treated subjects and their controls matched for height, weight and subscapular skinfold thickness

	Difference	S	n	Significance of the difference
S_1D_1 – Matched controls	−9 8	51 4	26	n s
S_4D_4 – Matched controls	+10 9	61 3	32[1]	n s
Treated subjects – Matched controls	⊥ 3 6	68 0	14	n s

[1]) Data from one of these subjects was missing

blood lipids and blood pressure have given different results Malmros *et al* (103) found no correlation between total serum cholesterol and systolic blood pressure in healthy Swedish males Waris (163) found a slight but not statistically significant difference in cholesterol between hypertensive patients and healthy controls Truedsson (160) in a sample of healthy subjects reported that after the elimination of the influence of age there were no significant correlations between plasma lipids and blood pressures for men For women on the other hand probably significant correlations persisted between the total lipids and lipoproteins respectively and systolic and diastolic blood pressure No significant correlation was found between the blood pressure in males or females and the increase in the plasma lipids 3 hours after ingestion of a fatty meal

Hood *et al* (119) studied the blood pressure in 500 hospital patients with hypercholesterolaemia They found that half of the subjects had elevated diastolic blood pressure and 60 per cent or 70 per cent (some difference between the two sexes) had levels in the upper quartile of the normal range for their age

The relationship between blood pressure and lipids has also been examined in population studies In the Framingham Study (76) there was a low correlation between systolic and diastolic blood pressure and cholesterol in males aged 45–62 (r=0 09) Diastolic blood pressure and cholesterol were significantly correlated in white males in Evans County after taking age into consideration No correlation was found between systolic blood pressure and cholesterol (100)

In two field surveys in Finland of men aged 40 to 59 a significant correlation was found between diastolic blood pressure and cholesterol in West Finland In East Finland there was no correlation between systolic and diastolic blood pressure and cholesterol (81)

In the present study blood lipids were studied in relation to blood pressure and age height weight and subscapular skinfold thickness were taken into consideration

The results from other studies and this study support the view that high blood pressure and serum cholesterol are independently related to the development of coronary artery disease

The relationship between triglycerides and blood pressure have been much less studied Hood *et al* studied a series of 458 patients selected on the basis of elevated serum cholesterol and they found no cor

TABLE 45 Differences in mean value of log 100 triglycerides (mMol/l) between S_1D_1 S_4D_4 treated subjects and their controls matched for height weight and subscapular skinfold thickness

	Difference	*S*	*n*	Significance of the difference
S_1D_1 – Matched controls	−0 10	0 31	26	n s
S_4D_4 – Matched controls	+0 0	0 19	3_[1])	$p<0.05$
Treated subjects – Matched controls	+0 09	0 39	14	n s

[1]) Data from one subject was missing

There is a close relationship between high blood pressure and vascular diseases. Myocardial infarction is one of the most common causes of death in hypertensive subjects. Atherosclerosis of the coronary arteries is the main etiological factor in myocardial infarction. It is a common belief that changes in lipid metabolism promote the development of atheroma.

Is the raised blood pressure the only factor which causes atheromatous vascular changes in hypertensive subjects or are lipid changes also present? In the present study blood lipids were studied in relation to blood pressure and in addition height, weight and skinfold thickness were taken into consideration (Appendix).

Results

A comparison of mean values of cholesterol and triglycerides between S_1D_1, S_2D_2, S_4D_4 and treated subjects is presented in table 42. The distribution of the triglycerides was skewed to the right and because of that the logarithmic value of original figures has been used. The differences in mean value of cholesterol between S_1D_1, S_4D_4 treated subjects and their controls matched for height, weight and subscapular skinfold are presented in table 44. There were no significant differences between the groups. Table 45 shows the differences in triglycerides between S_1D_1, S_4D_4 and treated subjects and their matched controls. There was a small but significant difference between S_4D_4 and the control group. No significant difference was found between S_1D_1 and treated subjects and their control groups.

Discussion

In the present series there were significantly higher triglycerides in subjects in S_4D_4 as compared to matched subjects. Cholesterol was not higher in S_4D_4 as compared to matched subjects. Previous investigations of the relationship between

TABLE 44 Differences in mean value of cholesterol (mg/100 ml) between S_1D_1, S_4D_4, treated subjects and their controls matched for height, weight and subscapular skinfold thickness

	Difference	S	n	Significance of the difference
S_1D_1 – Matched controls	−9 8	51 4	26	n s
S_4D_4 – Matched controls	−10 9	61 3	3-[1]	n s
Treated subjects – Matched controls	+ 3 6	68 0	14	n s

[1]) Data from one of these subjects was missing

There is a number of articles suggesting that the prevalence of high blood pressure in diabetic patients is higher than that in the population at large (29) (112) (127).

Few population studies have investigated the relationship between glucose tolerance and high blood pressure (121). In the present study it was possible to elucidate the following: Is fasting blood sugar increased in hypertensive subjects when age is eliminated and obesity is taken into consideration?

Results

A comparison of mean value of fasting blood sugar between S_1D_1, S_2D_2, S_4D_4 and treated subjects is presented in table 42. There was no significant difference between S_1D_1, S_4D_4 and treated subjects and their matched controls (table 46).

The number of subjects with fasting blood sugar ≥100 mg per 100 ml in S_3D_4, S_4D_3 and S_4D_4 was compared with the number of those among their matched controls. There were 10 subjects (16 per cent) in both categories.

Discussion

Fasting blood sugar was not increased in subjects in S_4D_4 as compared to matched controls. In the Tecumseh Study (121) the frequency of hyperglycemia in persons 40 years of age and older with diastolic hypertension was significantly greater than that in the over all population at a similar age. This discrepancy can be explained by the fact that in the present study the hypertensive subjects were of the same age and the same height, weight and subscapular skinfold thickness as the controls. Fasting blood sugar increases with age (115). In the present study fat subjects had a higher fasting blood sugar compared to non fat subjects (9).

Increased fasting blood sugar value can be used as a sign of impaired glucose tolerance (59) (116) (162).

Conn (29) has stated that it is possible that the frequency of aldosterone secreting

TABLE 46. Difference in mean value of fasting blood sugar (*mg/100 ml*) between S_1D_1, S_4D_4, treated subjects and their controls matched for height, weight and subscapular skinfold thickness

	Difference	*S*	*n*	Significance of the difference
S_1D_1 – Matched controls	−2.5	15.4	26	n.s.
S_4D_4 – Matched controls	−1.5	21.3	32[1]	n.s.
Treated subjects – Matched controls	−4.4	2[illegible].9	14	n.s.

[1] Data from one subject was missing

5

relation between triglycerides and blood pressure (119) Berkowitz (12) investigated the correlation between blood pressure and the fat tolerance curve, as measured by loading with labelled triolein in 50 patients, aged 29–63 years, with arterial hypertension He found an impaired fat tolerance curve in most of the hypertensive subjects The finding from the present study, that S_4D_4 had higher triglycerides compared to matched controls is of interest, but the relationship between triglycerides and blood pressure must be confirmed in other population studies before it is fruitful to speculate about elevated triglycerides as a factor in the relationship between high blood pressure and atherosclerosis

Smoking

That smoking has an immediate effect on the cardiovascular system has been recognized for many years Some of the effects are an increase in pulse rate slight increase in blood pressure increase in cardiac output and peripheral resistance and increased frequency of extrasystoles and ECG changes

The American Cancer Society has collected and published material relating mortality from several diseases to the habit of cigarette smoking (64) Cigarette consumption does not seem to underlie high blood pressure as it does coronary heart disease On the contrary Edwards *et al* (46) and Miall (107) in the United Kingdom and Karvonen in Finland (81) have found that smokers have lower pressures than non smokers Miall observed that pressures were highest among male ex smokers He attributed this finding to the increase in weight that usually followed cessation of smoking In this chapter the three variables smoking obesity and blood pressure have been analysed

Results

The subjects were divided into smokers, ex smokers smokers of 1–14 cigarette a day, 15 or more cigarettes a day and cigar and/or pipesmokers

Table 47 presents the prevalence rate of different smoking habits in B P I–IV The proportion of non smokers and ex smokers increased significantly with rising blood pressure On the contrary the proportion of smokers decreased with rising blood pressure It was significant only for subjects smoking 15 cigarettes or more a day If all smokers are taken together there was a significant decrease in prevalence rate of smokers with rising blood pressure Table 48 shows the prevalence rate of smoking habits in subjects in B P IV and in their matched controls It is clear that there were small differences between high blood pressure groups and controls when height weight and subscapular skinfold thickness were taken into consideration

Discussion

Previous studies have reported inte

TABLE 47 Prevalence rate of different smoking habits in B P I–IV

	B P I (n [illegible])	B P II (n 468)	B P III (n = 2[illegible]0)	B P IV (n = 7[illegible])	Probability
Non smokers	18 0%	[illegible]3 0%	22 5%	34 [illegible]%	χ^2 6 11 $p < 0$ 05
Ex smokers	13 4%	18 [illegible]%	[illegible]3 6%	23 3%	χ^2 6 [illegible] $p < 0$ 0[illegible]
1–14 cig	29 [illegible]%	[illegible]9 [illegible]%	23 5%	18 [illegible]%	χ^2 3 46 $p > 0$ 05
15– cig	28 1%	20 9%	15 5%	17 3%	χ^2 5 38 $p < 0$ 05
Pipe cigar	11 [illegible]%	8 6%	10 0%	4 0 %	χ^2 1 10 $p > 0$ 05

adenomas in subjects with high blood pressure of unknown cause might be as high as 20 per cent and he assumes that half of them have an impaired carbohydrate tolerance The number of subjects in the present study in B P IV with $\geq$100 mg/100 ml in fasting blood sugar was roughly of the same order (16 per cent) as the number expected from Conn's calculations (10 per cent) On the other hand, the frequency of subjects with $\geq$100 mg/100 ml in fasting blood sugar was the same as in the matched controls Results from this study could not confirm an increased prevalence of impaired glucose tolerance in subjects with high blood pressure

Despite the large number of published studies on high blood pressure usable data on demographic variables other than age and sex are few In the Evans County Study (99) slightly higher pressures were noted for the low social class Social class was evaluated using a modification of the Warner Scale which included occupation source of income and educational attainment of heads of household In the Framingham Study Dawber *et al* have noted that in the age group 50–59 years blood pressure was lower for persons with more education than in the remainder of this age group (36) This was also the finding in the U S Health Examination Survey (61)

Wilson s question number 6 from the Prague Symposium was What is the role of environmental factors such as geographical situation diet occupation and particularly mental strain or emotional stress? (166) Answering this question is difficult from a methodological point of view To obtain a nutritional history is time consuming and of questionable accuracy and was therefore deemed impossible to achieve in the present study In a modern urban society like that of Goteborg the number of different occupations is high In this sample of 855 men 259 different descriptions of the type of occupation were given A preliminary survey of the influence of occupation on blood pressure gave negative results and a more detailed investigation was deemed impracticable To measure mental strain and emotional stress in a meaningful way was not considered possible

In this chapter family income, rent, marital status, education and alcoholic problems as examples of more reliable socio-economic factors, are presented in relation to blood pressure

Methods

Information about the 1961 income of the subjects and their wives was acquired from the Revenue Office in Goteborg In this chapter the family income (subject s income plus wife s income) was studied

The subjects were asked about their monthly rent For home owners the rent was estimated by the subject

In the registers of the temperance board in Göteborg all alcoholic offenders since 1920 have been recorded The registrations were here used as a measurement of the presence of social complications of alcohol abuse

Higher education was defined as high school (realexamen) or above

Results

Table 49 shows the prevalence rate of married subjects subjects with alcoholic problems and subjects with higher education in B P I–IV There were no significant trends conserning rate of married subjects and subjects with higher education in relation to blood pressure A significant and positive trend was found in subjects with alcoholic problems in rela

resting but not always consistent differences between smokers and non smokers For example, Thomas (153) found that heavy smoking medical students are more often overweight than their fellows who smoke little or not at all, but the reverse is true among middle aged men in Finland (81) and in the U S A (17 a) Lundman (97) found in a study of monozygotic twins who were discordant with respect to smoking, that smokers showed lower basal systolic and diastolic blood pressure He explained the finding as a result of abstinence from smoking just before examination Blackburn *et al* (17 a) reported that smoking was associated with lower systolic and diastolic pressures and diminished diastolic pressure response to cold The relationship between blood pressure and smoking habits has been thoroughly examined in the large study of 17 populations of Keys *et al* (85) Non smokers tend to include an undue proportion of men with relatively high systolic blood pressure The excess of high systolic blood pressure among non smokers was reflected in a corresponding shortage of men with such high values among those men who always smoked 10 or more cigarettes a day The diastolic blood pressure distributions show the same trends as observed in regard to systolic blood pressure, but the departures from random expectation were even more marked In the study of Keys et al it was also found that non smokers tend to be fatter than smokers They discussed the possibility that the trend to higher blood pressure among non smokers can be dependent on this tendency They found that, when they ignored obesity, there was still an excessive frequency of subjects with high blood pressure among non smokers and they concluded that body fatness was not the explanation for their findings Either cigarette smoking tends to keep the blood pressure lower than it would otherwise be or non smokers are simply the kind of men who more often tend to become hypertensive In the present study there was found a clear relationship between smoking and blood pressure The proportion of smokers decreased with increase in blood pressure This is in agreement with most other studies in the field When obesity was taken into consideration there was the same frequency of smokers in B P IV as compared to matched controls From this it can be concluded that the tendency for non smokers to be found more often in high blood pressure groups can be explained by the body fatness

TABLE 48 Prevalence rate of smoking habits in subjects in B P IV and in their matched controls

	Non smoker	Ex smoker	1–14 cig	15–cig	pipe cigar
B P IV ($n=75$)	34 7%	25 3%	18 7%	17 3%	4 0%
Matched controls ($n=75$)	30 6%	21 3%	17 3%	21 3%	9 3%

SUMMARY

The present study is concerned with high blood pressure in a random sample of 50 year old men

Study population and methods

The study sample was from an urban population in a rapidly changing industrial society and consisted of men all born in the same year The important age factor was thereby controlled It was possible in a study of 855 men to obtain enough cases of all degrees of disease state from the malignant phase to the early case Representative control subjects were available among the non disease subjects There are several drawbacks to a cross-sectional study such as this one The selective effect of migration early death and non participation were studied Data presented showed that these factors did not influence the interpretation of the findings

One of the basic principles in research is that a sample must be chosen in such a way that it is possible to infer from results in the sample what the situation would be in the whole population In the present study an epidemiological approach has been used A representative sample of all men aged 50 of Goteborg has been studied with hospital examination techniques It is an attempt to combine the advantage of hospital and epidemiological study

Interobserver variation and diurnal changes have been eliminated by using one observer who always studied the subject at the same time of day

The casual blood pressure has been shown by previous investigators to be such a crude method that other manifestations of high blood pressure must be taken into consideration in a thorough going consideration of hypertensive disease In the present study two signs have been recorded as manifestations of hypertensive disease namely focal narrowings of arterioles and general attentuation of arterioles

Blood pressure

There was a close agreemet between the mean values of blood pressure in this study and other places scattered around the world From this it may be suggested that environmental factors are of only second order importance

Classifications of subjects

The extreme groups—high blood pressure and low blood pressure were of particular interest A suitable extreme group large enough for statistical handling consisted of about 5 per cent of the total sample of these men in both directions The cut off values for the extreme groups of systolic blood pressure were 175 mm Hg and 100 mm Hg and for diastolic blood pressure 115 and 70 mm Hg In order to reduce heterogeneity a combination of systolic and diastolic blood pressure was used The high blood pressure group

TABLE 49 Prevalence rate of married subjects subjects with alcoholic problems and subjects with higher education in B P I–IV

	B P I ($n=89$)	B P II ($n=468$)	B P III ($n=220$)	B P IV ($n=75$)
Married	79 7 %	87 0 %	83 6 %	84 0 %
Alcoholic problems	20 2 %	18 2 %	23 2 %	29 3 %
Higher education	18 0 %	19 4 %	11 4 %	16 0 %

tion to blood pressure ($\chi^2=4\ 24$ 1 D F $p<0\ 05$) The family income and monthly rent in B P I–IV are presented in table 50 No significant trends were found in these two variables in relation to blood pressure

Discussion

These results do not suggest that socio economic factors are important in relation to blood pressure This agrees with the finding that the mean value of blood pressure does not differ greatly between different white populations in spite of socio economic differences

There was a significant increase in subjects with alcoholic problems with rise in blood pressure in the present study In the Framingham Study (36) no significant effect on blood pressure was noted in any group in men and women The discrepancy in the findings is difficult to explain

Of importance in connection with socio economic factors is that the nonparticipation group consisted mainly of subjects from low income groups Unmarried individuals were common and subjects with alcoholic problems were frequent It is not likely that the non participation group has biased the blood pressure results

TABLE 50 Family income and monthly rent in B P I–IV

	B P I ($n=89$)	B P II ($n=468$)	B P III ($n=220$)	B P IV ($n=75$)
Family income (Sw Kr)				
< 10 000	19 1 %	12 4 %	15 [illegible] %	16 0 %
10 000–19 999	56 2 %	51 3 %	56 4 %	49 3 %
20 000–29 999	14 6 %	24 6 %	22 [illegible] %	26 [illegible] %
30 000–	10 1 %	11 8 %	[illegible] 5 %	8 0 %
	B P I ($n=88$)[1]	**B P II ($n=467$)[2]**	**B P III ($n=216$)[3]**	**B P IV ($n=75$)**
Monthly rent (Sw Kr)				
< 100	14 [illegible] %	7 [illegible] %	13 0 %	12 0 %
100–199	23 9 %	30 8 %	29 2 %	36 0 %
200–299	38 6 %	36 4 %	40 3 %	[illegible]6 [illegible] %
300–399	18 1 %	13 1 %	12 0 %	16 0 %
400–	4 6 %	12 2 %	[illegible] 6 %	9 3 %

1) Data for one subject was missing

2) Data for two subjects was missing

3) Data for four subjects was missing

was only one subject with history of renal disease (urinary calculi excluded) among the 47 subjects in S_4D_4 Significant differences in morning urine osmolality were found in S_4D_4 and treated subjects as compared to matched controls The explanation can be renal involvement with reduced concentration capacity and/or altered rhythm of water and solute excretion

Low blood pressure and infectious disease

There was a significant decrease in the number of subjects with pneumonia and pulmonary tuberculosis with rise in blood pressure The sedimentation rate was significantly higher in the low blood pressure group S_1D_1 as compared to the control group

The prevalence rate of calcifications in the hilar nodes and depressed diaphragms on x ray films of the lungs were decreased with increase in blood pressure Observations from this study and others indicate that infectious disease and nutritional status are of importance for the height of the blood pressure

Heart volume

Relative heart volume was greater in S_4D_4 compared to S_2D_2 On the other hand there was no significant difference in total heart volume when S_4D_4 was compared with a group matched for height weight and skinfold thickness

Lipids, uric acid and fasting blood sugar

When S_4D_4 and S_2D_2 were compared with matched controls concerning blood lipids uric acid and fasting blood sugar the only significant differences were found for S_1D_1 in uric acid and S_4D_4 in triglycerides S_1D_1 had a significant lower mean value of uric acid as compared to matched controls and S_4D_4 had a significantly higher mean value of triglycerides as compared to matched controls

Smoking habits

There was a clear relationship between smoking and blood pressure The frequency of smokers decreased with rising blood pressure The important factor seems to be body build since there was the same frequency of smoking habits in high blood pressure groups (B P IV) compared to body build matched controls

Socio economic factors

There was no significant trend in prevalence rate of married subjects and subjects with higher education in B P I-IV There was a significant increase in subjects with alcoholic problems with rise in blood pressure Family income and monthly rent were not related to blood pressure Thus although the non participant group consisted mainly of unmarried individuals in low income groups with alcoholic problems this seems unlikely to have influenced the blood pressure results

was defined as systolic blood pressure $\geq$175 mm Hg and diastolic blood pressure $\geq$115 (S_4D_4)

The low blood pressure group was defined as systolic blood pressure $\leq$110 mm Hg and diastolic blood pressure $\leq$70 mm Hg (S_1D_1) These two groups are compared with S_2D_2 (115–145/75–95) which was considered as an intermediate blood pressure group from which controls matched by height, weight and skinfold thickness were drawn The 14 subjects receiving antihypertensive treatment were considered as a separate group

High blood pressure

The single most characteristic sign of high blood pressure was focal narrowings in the arterioles of the eye ground In S_4D_4 the frequency of this finding was 64 0 per cent, compared to 0 6 per cent in S_2D_2

The blood pressure group S_4D_4 was, for reasons given, considered homogeneous enough to be generally called an entity on the basis of pressure level alone The number of subjects in this group was 33 In addition there were 14 under antihypertensive treatment The total prevalence of high blood pressure can be assessed at 5 5 per cent

Of the subjects with high blood pressure a third (14/47) was under treatment The majority of the treated subjects had a rather high casual blood pressure

In this age group elevated systolic blood pressure was as much associated with high blood pressure manifestations as elevated diastolic blood pressure

Obesity

The high blood pressure group S_4D_4 had significantly higher subscapular skin fold thickness and weight compared to S_2D_2 On the whole there seems to be agreement that the differences between intra arterial and cuff blood pressure are not generally affected by arm circumference, so that the relationship found between obesity and high blood pressure cannot therefore be explained by this factor The finding that the prevalence of subjects with hypertensive eye ground changes increased about three times with increase in subscapular skinfold and weight strongly support the assumption that the relationship between obesity and high blood pressure is a genuine one This relationship may be a direct one but the association may be the result of a common factor, for example, physical inactivity

In the further analysis the influence of obesity on the factors studied must be taken into consideration In order to eliminate as much as possible the influence of body build controls for all those subjects in groups S_1D_1 and S_4D_4 were selected and matched according to height weight and subscapular skinfold thickness

The fat hypertensive subjects had a higher frequency of cardiovascular manifestations of high blood pressure as compared to those of the non fat hypertensive subjects It can be concluded that the fat subjects with high blood pressure had as advanced or more advanced disease

Renal disease

A positive relation between frequency of urinary calculi and rise in blood pressure was found On the other hand there

S_1D_1 S_2D_4 S_4D_3 S_4D_4 and treated subjects							Controls from S_2D_2							
Subject	Weight (0,1 kg)	Height (cm)	Skinfold (0,1 mm)	Weight (S D units)	Height (S D units)	Skinfold (S D units)	Subject	Weight (0,1 kg)	Height (cm)	Skinfold (0,1 mm)	Weight (S D units)	Height (S D units)	Skinfold (S D units)	Geometric distance (S D units)
4	1006	178	215	2,246	0,497	0,989	917	972	177	208	1,937	0,331	0,877	0,368
24	748	178	89	-0,100	0,497	-1,023	101	746	178	87	-0,116	0,497	-1,055	0,037
27	727	172	130	-0,291	0,499	-0,368	132	740	172	143	-0,172	-0,499	-0,161	0,239
35	640	171	109	-1,082	-0,665	-0,703	112	627	172	92	-1,200	-0,499	-0,975	0,339
40	950	177	123	1,737	0,331	-0,480	647	910	177	153	1,373	0,331	-0,001	0,401
41	817	178	215	0,528	0,497	0,989	820	829	179	219	0,637	0,663	1,053	0,209
51	834	172	185	0,682	-0,499	0,510	36	866	173	177	0,919	-0,333	0,382	0,316
69	709	176	128	-0,454	0,165	-0,400	352	698	176	131	-0,554	0,165	0,352	0,111
83	859	182	171	0,910	1,161	0,286	574	851	182	177	0,837	1,161	0,382	0,120
84	695	177	131	-0,581	0,331	-0,352	600	676	177	126	-0,754	0,331	-0,432	0,190
90	694	178	95	-0,591	0,497	-0,927	33	691	178	90	-0,618	0,497	-1,007	0,084
93	620	175	83	-1,263	0,001	-1,119	679	628	176	74	-1,263	0,165	-1,262	0,220
135	811	177	135	0,473	0,331	-0,288	862	807	177	140	0,437	0,331	-0,209	0,088
141	894	178	206	1,228	0,497	0,845	752	909	178	206	1,364	0,497	0,845	0,136
144	722	179	82	-0,336	0,663	-1,135	566	732	179	86	0,245	0,663	-1,071	0,111
149	700	181	81	-0,536	0,995	-1,151	59	721	180	75	-0,345	0,829	-1,246	0,271
171	644	163	127	-1,045	-1,993	-0,416	833	646	163	131	-1,027	-1,993	-0,352	0,066
177	829	182	196	0,637	1,161	0,686	615	821	183	187	0,564	1,327	0,542	0,231
178	723	173	191	-0,327	-0,333	0,606	100	706	173	193	-0,481	-0,333	0,638	0,158
180	944	181	236	1,682	0,995	1,324	11	900	182	217	1,282	1,161	1,021	0,529
188	867	184	242	0,982	1,493	1,420	727	872	182	238	1,028	1,161	1,356	0,341
192	725	177	81	-0,309	0,331	-1,151	279	718	178	81	-0,372	0,497	-1,151	0,178
212	834	173	171	0,682	0,333	0,286	533	828	173	171	0,555	-0,333	0,286	0,127
216	751	168	185	-0,072	-1,163	0,510	899	760	168	197	0,009	-1,163	0,702	0,208
218	751	180	186	-0,072	0,829	0,526	516	769	180	172	0,091	0,829	0,302	0,277
240	1235	182	394	4,328	1,161	3,847	817	995	188	263	2,146	2,157	1,755	3,182
257	1029	178	278	2,455	0,497	1,995	982	978	178	299	1,982	0,497	2,330	0,572
260	742	176	293	-0,154	0,165	2,234	361	799	175	266	0,364	-0,001	1,803	0,694
262	779	188	117	0,182	2,157	-0,576	170	759	188	103	0,000	2,157	-0,799	0,288
278	843	175	199	0,764	-0,001	0,733	167	842	175	202	0,755	0,001	0,781	0,049
288	1032	175	271	2,483	-0,001	1,883	76	941	177	258	1,655	0,331	1,675	0,915
305	613	171	64	1,327	0,665	-1,422	442	613	169	75	-1,327	-0,997	-1,246	0,376
313	1035	179	255	2,510	0,663	1,628	969	953	176	202	1,764	0,165	0,781	1,233
345	883	172	296	1,128	-0,499	2,282	298	949	172	301	1,728	-0,499	2,362	0,805
346	694	173	205	-0,591	-0,333	0,829	890	716	171	195	-0,391	-0,665	0,670	0,419
351	720	176	147	-0,354	0,165	-0,097	197	708	175	143	-0,463	-0,001	-0,161	0,209
357	703	159	149	-0,509	-2,657	-0,065	213	727	162	147	-0,291	2,159	-0,097	0,545
398	561	164	64	-1,800	-1,827	-1,422	773	591	165	97	-1,527	-1,661	-0,895	0,616
401	836	180	189	0,700	0,829	0,574	56	825	179	191	0,600	0,663	0,606	0,196
419	614	176	107	-1,318	0,165	-0,735	559	620	175	106	-1,263	-0,001	-0,751	0,175
421	677	167	183	-0,745	-1,329	0,478	256	697	164	177	-0,563	-1,827	0,382	0,539
427	891	190	141	1,201	2,489	0,193	530	864	188	155	0,955	2,157	0,031	0,470
431	1015	183	377	2,328	1,327	3,575	722	1100	182	327	3,101	1,161	2,777	1,123
443	899	178	235	1,273	0,497	1,308	558	896	177	222	1,246	0,331	1,101	0,267
481	891	169	265	1,701	-0,997	1,787	178	901	173	254	1,291	-0,333	1,612	0,493
482	631	183	72	1,163	1,327	-1,294	461	624	184	70	-1,227	1,493	-1,326	0,181
518	979	180	188	1,546	0,829	0,558	907	933	180	183	1,582	0,829	0,478	0,088
519	592	169	93	-1,518	0,997	0,959	723	581	169	100	-1,618	0,997	-0,847	0,150
523	731	176	105	0,254	0,165	0,767	444	721	176	107	-0,345	0,165	-0,735	0,896
531	839	173	155	0,646	-0,333	0,031	219	820	172	160	0,555	0,499	0,111	0,205

APPENDIX

In order to eliminate the important influence of obesity and overweight on factors investigated in this study, controls matched by height, weight, and skinfold thickness for subjects in S_1D_1, S_3D_4, S_4D_3, S_4D_4 and treated subjects have been drawn from the group with intermediate blood pressure (S_2D_2)

Of the 468 subjects in S_2D_2, data were missing for 45 subjects concerning some of the factors aortic breadth, heart volume in standing and prone position, total serum protein, serum albumin, height, weight and subscapular skinfold thickness They were excluded In the proband group one subject was excluded because of missing data The absolute values for weight, height and subscapular skinfold were transformed to S D units The values of the probands and the controls were placed in a coordinate system in three dimensions A control subject was chosen for every proband in such a way that the geometric distance between the proband and the control was as short as possible This distance was an assessment of the error of the method In 93 pairs of proband and control the distance was less than one S D unit Of the seven remaining pairs the distance was between 1 S D – 3 S D in 5 pairs and between 3–4 S D in two pairs The analysis was done in a Saab D 21 computer

S_1D_1 S_3D_4 S_4D_3 S_4D_4 and treated subjects

Subject	Weight (0 1 kg)	Height (cm)	Skinfold (0 1 mm)	Weight (S D units)	Height (S D units)	Skinfold (S D units)
4	1006	178	215	2 246	0 497	0 989
24	748	179	89	-0 100	0 497	-1 023
27	727	172	130	-0 291	-0 499	-0 768
35	640	171	109	-1 082	-0 665	-0 703
40	958	177	123	1 737	0 331	-0 480
41	917	178	215	0 529	0 497	0 989
51	834	172	185	0 682	-0 499	0 510
69	759	176	128	-0 454	0 165	-0 400
83	850	182	171	0 910	1 161	0 286
84	695	177	131	-0 581	0 331	-0 352
90	694	178	95	-0 591	0 497	-0 927
93	620	175	83	-1 263	-0 001	-1 119
135	8 1	177	139	0 473	0 331	-0 788
141	894	178	206	1 228	0 497	0 845
144	722	179	82	-0 336	0 663	-1 135
149	700	181	81	-0 536	0 995	-1 151
171	644	163	127	-1 045	-1 993	-0 416
177	829	182	196	0 637	1 161	0 686
176	723	173	191	0 327	0 333	0 606
180	944	181	236	1 482	0 995	1 324
186	867	184	242	0 982	1 493	1 420
192	725	177	81	-0 309	0 331	-1 151
212	834	173	171	0 682	0 333	0 286
216	751	168	185	0 072	1 163	0 510
218	751	180	186	-0 072	0 829	0 526
240	1235	182	394	4 328	1 161	3 847
257	1829	178	278	2 455	0 497	1 995
260	742	176	293	0 154	0 165	2 234
62	779	188	117	0 182	2 157	-0 576
278	843	175	199	0 764	0 001	0 733
288	1032	175	271	2 483	-0 001	1 887
305	613	171	64	-1 327	-0 665	-1 422
313	1035	179	255	2 510	0 663	1 628
345	883	172	296	1 128	-0 499	2 282
346	694	173	205	-0 591	-0 333	0 829
351	720	176	147	-0 354	0 165	-0 697
357	703	159	149	-0 509	-2 657	-0 865
399	561	164	64	-1 808	-1 827	-1 422
401	836	180	189	0 700	0 829	0 574
415	614	176	107	1 318	0 165	-0 739
421	677	167	183	-0 745	-1 329	0 478
427	891	190	141	1 201	2 489	0 193
431	1015	183	377	2 328	1 327	3 575
443	899	178	235	1 273	0 497	1 308
481	891	169	265	1 701	-0 997	1 787
482	631	163	72	-1 163	1 327	-1 294
518	979	180	188	1 946	0 829	0 558
519	892	169	93	-1 518	-0 997	-0 959
523	731	176	105	0 254	0 165	-0 767
531	830	173	155	0 646	-0 333	0 031

Controls from S_2D_2

Subject	Weight (0,1 kg)	Height (cm)	Skinfold (0 1 mm)	Weight (S D units)	Height (S D units)	Skinfold (S D units)	Geometric distance (S D units)
917	972	177	208	1 937	0 331	0,877	0 368
101	746	178	87	0 118	0 497	-1,055	0 037
132	740	172	143	-0 172	-0 499	-0,161	0 239
112	627	172	92	-1 200	-0 499	-0,975	0 339
647	910	177	153	1 373	0 331	-0,001	0 401
820	829	179	219	0 637	0 663	1 053	0 209
36	860	173	177	0 919	-0 333	0,382	0 316
352	698	176	131	-0 594	0 165	-0,352	0 111
574	851	182	177	0 837	1 161	0 382	0 120
600	676	177	126	-0 794	0 331	-0 432	0 190
33	691	178	90	-0 618	0 497	-1,007	0 084
679	620	176	74	-1 263	0 165	-1 262	0 220
862	807	177	140	0 437	0 331	-0,209	0 088
752	909	178	206	1 364	0,497	0 845	0 136
566	732	179	86	-0 245	0 663	-1 071	0 111
[illegible]	721	180	75	-0 345	0 829	-1 246	0 271
833	646	163	131	-1 027	-1 993	0 352	0 066
615	821	183	187	0 564	1 327	0,542	0 231
100	706	173	193	-0 481	-0 333	0,638	0 158
11	900	182	217	1 282	1 161	1,021	0 529
727	872	182	238	1 028	1 161	1,356	0 341
279	718	178	81	-0 372	0,497	-1,151	0 178
533	820	173	171	0 555	-0,3.3	0,286	0 127
499	760	168	197	0 809	-1 163	0 702	0,208
516	769	180	172	0 091	0 829	0 302	0 277
817	995	188	263	2 166	2 157	1,755	3 182
982	978	178	299	1 992	0 497	2,330	0 572
361	799	175	266	0 364	-0 001	1,803	0 694
170	759	188	103	0 000	2 157	-0 799	0 288
167	842	175	202	0 755	0 001	0 781	0 049
76	941	177	258	1 455	0 331	1 675	0 919
442	613	169	75	-1 327	-0 997	-1,246	0 374
969	953	176	202	1 764	0 165	0,781	1 233
298	949	172	301	1 728	0 499	2,362	0 605
890	716	171	195	-0 391	-0 665	0,670	0 419
197	708	175	143	-0 665	-0 001	-0 161	0 209
213	727	162	147	-0 291	-2 159	-0 097	0 545
773	591	165	97	-1 527	-1 661	-0,895	0 616
56	825	179	191	0 600	0 663	0,606	0 196
559	620	175	186	-1 263	-0 001	-0,751	0 175
256	697	164	177	0 563	-1 827	0,382	0 939
530	864	188	155	0 955	2 157	0,031	0 470
722	1100	182	327	3 101	1 161	2,777	1 123
558	896	177	222	1 246	0 331	1,101	0 267
128	901	173	254	1 291	-0,333	1 612	0 693
461	624	184	70	-1 227	1 493	-1 326	0 181
907	933	180	183	1 582	0 829	0,478	0 088
723	581	169	100	-1 618	0 997	-0,847	0 150
444	721	176	107	-0 345	0 165	-0 735	0 096
219	820	172	160	0 555	-0 499	0 111	0 205

APPENDIX

In order to eliminate the important influence of obesity and overweight on factors investigated in this study, controls matched by height, weight, and skinfold thickness for subjects in S_1D_1, S_3D_4, S_4D_3, S_4D_4 and treated subjects have been drawn from the group with intermediate blood pressure (S_2D_2)

Of the 468 subjects in S_2D_2, data were missing for 45 subjects concerning some of the factors aortic breadth, heart volume in standing and prone position, total serum protein serum albumin, height, weight and subscapular skinfold thickness They were excluded In the proband group one subject was excluded because of missing data The absolute values for weight, height and subscapular skinfold were transformed to S D units The values of the probands and the controls were placed in a coordinate system in three dimensions A control subject was chosen for every proband in such a way that the geometric distance between the proband and the control was as short as possible This distance was an assessment of the error of the method In 93 pairs of proband and control the distance was less than one S D unit Of the seven remaining pairs the distance was between 1 S D–3 S D in 5 pairs and between 3–4 S D in two pairs The analysis was done in a Saab D 21 computer

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Mr Bengt Perbo M S took charge of the computer analysis of the data

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S_1D_1 S_2D_4 S_4D_3 S_4D_4 and treated subjects

Subject	Weight (0,1 kg)	Height (cm)	Skinfold (0,1 mm)	Weight (S D units)	Height (S D units)	Skinfold (S D units)
542	785	173	156	0,237	-0 333	0 047
575	900	17A	326	1 282	0 497	?,761
582	880	177	337	1 101	0 331	2 857
606	701	170	93	-0 527	-0 831	-0 959
608	633	178	76	-1 145	0 497	-1,230
611	503	162	75	-2,327	-2 159	-1 246
619	A18	167	131	0 537	-1 329	-0,352
621	823	173	185	0 582	-0 333	0 510
641	752	177	314	-0 063	0 331	2 569
643	A64	168	353	0 955	-1 163	3,192
656	1037	179	394	2 528	0 663	3 847
672	700	171	139	-0 536	-0 665	-0,224
681	A53	173	136	0 855	-0 333	-0,272
696	753	183	110	-0 854	1 327	-0 6A7
717	816	174	343	0 519	-0 167	3 032
724	687	173	106	-0 654	-0 333	-0 751
728	859	1A4	136	0 910	1 493	-0 272
729	9A4	182	299	2 046	1 161	2,330
738	820	191	152	0,555	2 655	-0 017
755	582	178	74	-1 609	0 497	-1,262
763	501	167	72	-2,345	-1 329	-1,294
770	767	170	156	0 073	-0 831	0 047
785	810	168	163	0 464	-1 163	0,159
786	678	164	253	-0 736	-1 827	1 596
790	820	169	190	0 555	-0 997	0,590
819	987	.79	173	2,073	0 663	0,318
824	811	181	157	0,473	0 995	0,063
830	732	173	113	-0,245	-0 333	-0,640
846	651	163	211	-0 982	-1 993	0,925
855	718	160	182	-0 372	-2 491	0,462
856	643	170	137	-1 054	-0 831	-0,256
873	578	170	61	-1 645	-0 831	-1 470
875	578	163	185	-1 645	-1 993	0 510
877	835	171	193	0 691	-0 665	0 638
883	696	180	99	-0 572	0 829	-0 863
887	653	182	53	-0 963	1 161	-1,598
892	773	182	95	0 128	1 161	-0 927
893	782	175	128	0 210	-0 001	-0 400
895	1190	179	397	3 919	0 663	3 895
903	918	180	284	1 446	0 829	2 091
905	840	178	154	0 737	0 497	0 015
914	680	171	107	-0 718	-0 665	-0 735
919	833	183	10A	0 673	1 327	-0 719
928	569	166	83	-1 727	-1 495	-1 119
933	713	169	167	-0 418	-0 997	0 223
946	606	174	76	-1 391	-0 167	-1 230
955	741	175	166	-0 163	-0 001	0,207
964	647	172	148	-1 018	-0 499	-0 081
968	743	175	134	-0 145	-0 001	-0 304
1005	646	173	96	-1 027	-0 333	-0 911

Controls from S_2D_2

Subject	Weight (0,1 kg)	Height (cm)	Skinfold (0,1 mm)	Weight (S D units)	Height (S D units)	Skinfold (S D units)	Geometric distance (S D units)
776	798	172	147	0,355	-0,499	-0,097	0 249
68	956	177	345	1 792	0,331	3,064	0 615
258	867	179	345	0 982	0,663	3,064	0 409
568	699	171	104	-0,545	-0,665	-0,783	0 242
685	631	178	74	-1,163	0,497	-1,262	0 037
60	466	163	85	-2,664	-1,953	-1,087	0 408
663	789	169	143	0,273	-0,997	-0,161	0,465
980	835	174	185	0 691	-0,167	0,510	0 199
570	835	174	284	0 691	-0,167	2,091	1 023
224	706	164	367	-0,481	-1,827	3,416	1 598
133	1082	190	307	2 937	2,489	2,458	2,331
732	693	171	132	-0,600	-0,665	-0,336	0 129
636	851	175	151	0,837	-0 001	-0,033	0 410
227	740	183	118	-0,172	1,327	-0,560	0 174
136	871	174	346	1 019	-0,167	3,080	0 502
308	681	173	105	-0 709	-0,333	-0,767	0,057
98	847	185	147	0 801	1,659	-0,097	0 265
908	908	182	257	1 355	1,161	1,659	0 963
538	833	186	169	0,673	1 825	0,254	0 881
603	549	178	74	-1,909	0 497	-1,262	0 300
669	580	168	90	-1,627	-1,163	-1,007	0,791
131	755	170	158	-0,036	-0 831	0,079	0 114
365	829	170	165	0,637	-0,831	0,191	0 376
501	652	164	273	-0 972	-1,827	1,915	0 397
492	820	169	207	0 555	-0,997	0,861	0 271
493	939	178	192	1 637	0,497	0,622	0 557
154	783	180	149	0 219	0,829	-0,065	0 330
220	740	172	123	-0 172	-0,499	-0,480	0 242
647	680	162	218	-0 718	-2 159	1,037	0 331
259	725	162	179	-0,309	-2,159	0,414	0 341
74	640	169	139	-1 082	-0,997	-0,224	0,171
471	577	168	74	-1 654	-1 163	-1,262	0 392
342	649	161	186	-1,000	-2,325	0,526	0 726
194	845	174	205	0 782	-0,167	0,829	0,541
383	703	179	107	-0 509	0,663	-0,735	0 219
376	631	182	85	-1 163	1,161	-1,087	0 549
429	776	182	109	0 155	1 161	-0,703	0 229
440	780	175	130	0 191	-0 001	-0,368	0 037
437	956	185	214	1,792	1 659	0,973	3 749
1009	900	181	258	1 282	0,995	1,675	0 478
920	845	178	160	0 782	0 497	0,111	0 108
327	683	171	106	-0 691	-0 665	-0,751	0 032
244	805	182	111	0 419	1 161	-0,672	0 308
719	605	166	80	-1 400	-1,495	-1,166	0 331
528	695	169	169	-0 581	-0 997	0,254	0 167
612	601	174	76	-1 436	-0 167	-1,230	0 845
148	738	174	155	-0 191	-0 167	0 031	0 243
947	673	172	149	-0 782	-0 499	-0 129	0 241
901	752	175	136	-0 063	-0 001	-0 272	0 088
107	663	173	85	-0 872	-0 333	-1,087	0 234

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SUPPLEMENTUM 471

PLATELETS, COAGULATION FACTORS AND 5-HYDROXYTRYPTAMINE IN SENSITIZATION, ANAPHYLAXIS AND BURN INJURIES

Effect of Heparin and Possible Relation to Intravascular Coagulation

by

STIG-ARNE JOHANSSON

STOCKHOLM 1967

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From the Department of Medicine (Head H. Lagerlof M.D.), Karolinska Sjukhuset Medical Service VI (Head I. G. Porjé M.D.) Sodersjukhuset and Coagulation Research Department (Head B. Blomback, M.D.) Wallenberg laboratories, Stockholm, Sweden.

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I On the protective action of heparin in cases of anaphylactic shock
Acta Dermatovenereol 41 500 1961

II Studies on platelets and heparin during sensitization and anaphylaxis
Acta Allergol XIX, 142—162 1964

III Coagulation factors and defibrination syndrome in anaphylaxis
Acta physiol scand in press In collaboration with M Blombäck and H E Sjoberg

IV Inhibition of thrombocytopenia and 5 hydroxytryptamine release in anaphylactic shock by heparin
Acta physiol scand 50 95—104 1960

V Influence of heparin on thrombocytopenia in allergic reactions
Acta Med Scand 168 165—168 1960 In collaboration with A Lundberg and H E Sjoberg

VI Studies on blood coagulation factors in a case of liver cirrhosis Remission of the hemorrhagic tendency on treatment with heparin
Acta Med Scand 175 177—183 1964

VII 5 hydroxytryptamine in burns
Acta physiol scand 48 126—132 1960

VIII Heparin and thrombocytopenia in experimental burn injuries
Acta physiol scand 53 239—246 1961

IX Heparin and some coagulation factors in experimental burn injuries
Acta chir scand 127 346—349 1964

References to these papers will be made by using the above Roman figures

CONTENTS

The anaphylactic reaction was first described by Richet (1902) in dogs given sublethal doses of toxic extracts of the tentacles of sea anemones actinias and mussels He noted only mild reactions in normal dogs but when they received a similar injection *about 3 weeks later violent* symptoms appeared in the form of dyspnoea, vomiting salivation, general weakness diarrhoea and a marked fall in blood pressure followed by death He named the phenomenon *anaphylaxie*

In 1903 Arthus showed that Richet's phenomenon could be elicited by repeated injections of non toxic material such as serum protein or egg albumin In the same year von Pirquet & Schick observed the importance of the time interval for the anaphylactic manifestations when heterologous proteins were repeatedly administered to human subjects

It was early shown that a large number of antigens were able to produce anaphylactic symptoms Moreover each animal species showed a uniform pattern of reactions to different antigens These facts indicated that *the reactions were unspecific* with respect to the nature of the antigen

Concurrently with the accumulation of a wide variety of experimental observations on the conditions under which an anaphylactic reaction is elicited numerous theories were advanced to explain the manifestations (see Dragstedt 1941 Rocha e Silva 1959)

The definition by Dragstedt (1941) of anaphylaxis as an autointoxication by physiologically active substances normally resident in various tissue cells and liberated therefrom by some change in cellular permeability brought about by antigen antibody reaction" is now generally accepted

The main characteristics of anaphylactic shock in most animal species includes con*tractions of smooth muscles* oedema formation due to increased capillary permeability, and a fall in blood pressure

The important role of histamine in anaphylaxis was indicated already by Dale & Laidlaw (1910)

Heparin and anaphylaxis

The mast cells contain histamine heparin and 5 hydroxytryptamine They are disrupted during anaphylaxis Although many details in this chain of events are elucidated (*cf* Uvnäs 1965) their role in the production symptoms and signs has not yet been clarified (*cf* Riley 1959)

A hypothesis on the physiological role of heparin was put forward by Mellanby (1934) This hypothesis was formulated by Jaques Bell & Cho (1954) as follows

> Heparin is secreted locally by the mast cells to prevent intravascular clotting due to entry of thromboplastin into the blood following tissue damage What we know about heparin fits this plausibility — *very rarely is any amount found* in the general circulation on injection, it is removed rapidly from the circulation it is found in the mast cells close to blood vessels and in greatest amount and number where there is *extensive strain* (lung) or rapid breakdown of tissue (involuting thymus area of invasion by tumors) However up to date, satisfactory direct evidence has eluded us It *may* be *of course*, that heparin is associated with other functions.

as on the local Arthus phenomenon They found that some compounds were highly active against such shock, especially poly saccharides *from gram negative* bacteria Vegetable polysaccharides displayed this effect to a much lesser extent

Since the clinical application of heparin in thrombotic and arteriosclerotic disease are well known they are not summarized here (*cf* Jorpes 1946 Engelberg 1963) Heparin treatment with good results in man has been reported in pemphigus (Magner Man son & Pepple 1951) local pyogenic infec tions (Sandblom, Ekstrom & Quist 1947) acute rheumatic polyarthritis (Wassen & Zander 1945) peritendinitis crepitans (Rais 1954 Kvist 1957) frostbite (Lange Weiner & Boyd 1947) burns (Stoker 1951 Berberian 1960 Liljedahl 1967) acquired haemolytic anaemia (Owren 1949 MacFar land Galbraigh & Miale 1960 Pas & Mon to 1966) obstetric shock (Schneider 1959) and multiple sclerosis (Courville 1959)

Platelets and anaphylaxis

It was observed *in vitro* by Marino (1905) and *in vivo* by le Sourd & Pagniez (1907) and Achard & Aynaud (1908) that the pla telets of the rabbit and horse immunized *with serum from the guinea pig and dog* were agglutinated by serum used for sen sitization

The antigenic specificity of blood plate lets has been well established Platelets are agglutinated by a corresponding antiserum and in the presence of complement undergo lysis (Lee & Robertson 1916) Sensitized platelets are phagocytized by leukocytes and monocytes (Miescher 1953) Another kind of immunogenic platelet damage can be produced by antibody reaction with a variety of antigens unrelated to platelets which only indirectly results in platelet damage (Humphrey & Jaques 1955 Nelson & Nel son 1959) Platelets may also combine with haptene and thus give rise to a new platelet and haptene dependent antigen The cor responding antiserum reacts only with the combined antigen This mechanism has been *postulated for a variety of allergic disorders* involving platelets leukocytes and red cells (Ackroyd 1955)

Rocha e Silva (1950) recorded for the first time that platelets could not only be agglutinated by antigen antibody reaction, but were in addition profoundly damaged and lysed in the capillaries Abell & Schenk (1938) found that injections of antigen provoked a marked contraction of the ar terioles with clumping of cells adhering to the vascular endothelium In some instances leukocytic emboli were formed which blocked the circulation in the capillaries and veins

Although Eagle Johnston & Ravdin (1927) observed no regular changes in plate *lets during anaphylaxis Kopeloff & Kope* loff (1941) were able to show a decrease in the number of platelets Furthermore they demonstrated a definite correlation between the severity of anaphylactic shock and the *decrease in platelet count*

Platelets in blood coagulation and haemostasis

The role of platelets in arresting bleeding was pointed out by Hayem (1882) and was confirmed by Duke (1912) The pla telets key position in normal haemostasis has been verified by many authors (*cf* Roskam *et al* 1959)

As early as 1865 Schulze observed that

I am, however, inclined to believe rather that it has been lack of methods suitable for testing Mellanby's hypothesis which has been responsible for negative results. With greater knowledge of the complexity of the nature of the heparins it will be possible to design adequate experiments to test their physiological significance.

Heparin in varying concentrations binds and inhibits the activity of many enzymes, *e g* thrombin trypsin, pepsin, elastase, α-amylase, fumarase, ribonuclease, β-glucuronidase, acid and alkaline phosphatase, and the phosphomonoesterase of red blood cells (*cf* Jorpes 1946, Engelberg 1963). Heparin also decreases the toxicity of toxins and drugs, *e g* cobra venom, bee venom, coagulant snake venoms viper venom, bacterial endotoxin, digitalis, compound 48/80, curare, tubocurarine, the antibiotics polymyxin B neomycin, viomycin, dihydrostreptomycin and cyanide (*cf* Engelberg 1963). The combination with heparin decreases the toxicity of the antibiotic drugs without affecting their antibacterial potency (Karasek & Mourek 1959). It also protects rabbits against inoculation with *Sanarelli* myxoma virus (Thiery 1953).

In addition, heparin affects cellular permeability (Capraro Marro & Valzelli 1958) and inhibits the uptake of serum bound lipids by arterial endothelial cells (Lazzarini Robertson 1961). Heparin further minimizes the adherence of platelets to the endothelial line following endothelial damage (Samuels & Webster 1952). The action of heparin on the blood lipids first noted by Hahn (1943) is well documented (*cf* Engelberg 1963).

A striking decrease in the coagulability of dog blood during anaphylaxis was noted by Biedl & Kraus (1909). Eagle Johnston & Ravdin (1937) showed that this impaired coagulability is not due to a deficiency of fibrinogen, platelets or prothrombin. Instead, they found increased activity of an antithrombic substance in dog plasma. In 1941, Jaques & Waters showed this compound to be heparin. The physiological importance of heparin in anaphylaxis has not been penetrated. Kyes & Strauser (1926) and Williams & van der Carr (1927) maintained that pretreatment with heparin protected sensitized pigeons and guinea pigs from shock. Several authors were, however unable to confirm this observation (Hanzlik, Butt & Stockton 1927, Hyde 1927, Loewenthal 1927, Reed & Lamson 1927, Waud 1927, and Reed 1930).

In 1940 Silfverskiold observed that pretreatment of sensitized rabbits with heparin prevented or diminished the glomerulonephritic reaction to a third horse serum injection. Heparin inhibits the Arthus phenomenon (Gregoire 1946), uveitis following intraocular injection of antigen in hypersensitized rabbits (Bick & Wood 1950) and local and generalized Schwartzman reactions in rabbits (Good & Thomas 1953). Gentele & Holmgren (1951) showed that heparin is also partially effective in relieving cutaneous allergic reactions.

Dragstedt Wells & Rocha e Silva (1942) found that heparin inhibited the release of histamine into plasma from the blood cells of rabbits after addition of trypsin (0.5 mg/ml). Heparin also inhibited the release of histamine when antigen was added to blood of sensitized rabbits. The amount of heparin necessary was much greater than that necessary to prevent visible coagulation.

Meier Beim & Jaques (1956, 1957) investigated the effect of many other polysaccharides on anaphylactic shock, as well

as on the local Arthus phenomenon They found that some compounds were highly active against such shock, especially poly saccharides from gram negative bacteria Vegetable polysaccharides displayed this effect to a much lesser extent

Since the clinical application of heparin in thrombotic and arteriosclerotic disease are well known they are not summarized here (*cf* Jorpes 1946 Engelberg 1963) Heparin *treatment* with good results in man has been reported in pemphigus (Magner Man son & Pepple 1951) local pyogenic infec tions (Sandblom, Ekstrom & Quist 1947) acute rheumatic polyarthritis (Wassen & Zander 1945) peritendinitis crepitans (Rais 1954 Krist 1957) *frostbite* (Lange Weiner & Boyd 1947) burns (Stoker 1951 Biberian 1960 Liljedahl 1967) acquired haemolytic anaemia (Owren 1949 MacFar land Galbraigh & Miale 1960 Pas & Mon to 1966) obstetric shock (Schneider 1959) and multiple sclerosis (Courville 1959)

Platelets and anaphylaxis

It was observed *in vitro* by Marino (1905) and *in vivo* by le Sourd & Pagniez (1907) and Achard & Aynaud (1908) that the pla telets of the rabbit and horse immunized with serum from the guinea pig and dog were agglutinated by serum used for sen sitization

The antigenic specificity of blood plate lets has been well established Platelets are agglutinated by a corresponding *antiserum* and in the presence of complement undergo lysis (Lee & Robertson 1916) Sensitized platelets are phagocytized by leukocytes and monocytes (Miescher 1953) Another kind of immunogenic platelet damage can be produced by antibody reaction with a variety of antigens unrelated to platelets, which only indirectly results in platelet damage (Humphrey & Jaques 1955 Nelson & Nel son 1959) Platelets *may* also combine with haptene and thus give rise to a new platelet and haptene dependent antigen The cor responding antiserum reacts *only* with the combined antigen This mechanism has been postulated for a variety of allergic disorders involving platelets leukocytes and red cells (Ackroyd 1955)

Rocha e Silva (1950) recorded for the first time that platelets could not only be agglutinated by antigen antibody reaction but were in addition profoundly damaged and lysed in the capillaries Abell & Schenk (1938) *found that injections of antigen* provoked a marked contraction of the ar terioles with clumping of cells adhering to the vascular endothelium In some instances leukocytic emboli were formed which blocked the circulation in the capillaries and veins

Although Eagle Johnston & Ravdin (1927) observed no regular changes in plate lets during anaphylaxis Kopeloff & Kope loff (1941) were able to show a decrease in the number of platelets Furthermore they demonstrated a definite correlation between the severity of anaphylactic shock and the decrease in platelet count

Platelets in blood coagulation and haemostasis

The role of platelets in arresting bleeding was pointed out by Hayem (1882), and was confirmed by Duke (1912) The pla telets key position in normal haemostasis has been verified by many authors (*cf* Roskam *et al* 1959)

As early as 1865, Schulze observed that

I am, however inclined to believe *rather that* it has been lack of methods suitable *for testing* Mellanby s hypothesis which has been *responsible for negative results* With greater knowledge of the *complexity* of the nature of the heparins it will be *possible* to design adequate experiments to test their *physiological significance*

Heparin in varying concentrations binds and inhibits *the* activity *of* many enzymes, *e g* thrombin, trypsin, pepsin, elastase, *α amylase, fumarase,* ribonuclease, *β* glucuronidase, acid and alkaline phosphatase, and the phosphomonoesterase of red blood cells (*cf* Jorpes 1946, Engelberg 1963) Heparin also decreases the toxicity of toxins and drugs, *e g* cobra venom bee venom, coagulant snake venoms, viper venom, bacterial endotoxin, digitalis, compound 48/80, curare, tubocurarine, the antibiotics, polymyxin B, neomycin, viomycin, dihydrostreptomycin and cyanide (*cf* Engelberg 1963) The combination with heparin decreases the toxicity of the antibiotic drugs without affecting their antibacterial potency (Karasek & Mourek 1959) It also protects rabbits against inoculation with *Sanarelli* myxoma virus (Thiery 1953)

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sphere The rapid swelling (within 30 seconds) *increases* their volume by about 26 per cent and probably represents an explosive uptake of water (Bull & Zucker 1965)

In vitro aggregates formed by thrombin show active contraction most likely due to activation of thrombostenin This contractile protein of the platelets may play an important role in consolidation of the haemostatic plug (Bettex Galland & Lüscher 1965)

Fibrinogen and related proteins on the surface of the platelets seem to be essential for platelet aggregation by thrombin After treatment with proteolytic enzymes to deplete the platelets of fibrinogen no aggregation occurs after addition of thrombin (Morse Jackson & Conley 1965) Niewiarowski & Thomas (1966) noted however that the platelet aggregation by thrombin is not related to fibrinogen fibrin formation

Gugler & Luscher (1963) nevertheless found that the platelets of a patient with afibrinogenaemia, lacking any traces of immunologically detectable fibrinogen, were aggregated by thrombin

The findings that toluene sulphonyl arginine methyl ester (TAMe) and a number of other esters of arginine and lysine inhibit *ADP aggregation indicate that* an *enzyme* with esterase activity may be involved (Salzman & Chambers 1964) Cocaine and charged amphipathic compounds such as cetylpyridinium bromide inhibit all types of aggregation Metabolic inhibitors such as cyanide fluoride and azide do not inhibit clumping by thrombin ADP or 5 HT in concentrations of 2 5 or 10×10^{-4} M (Mitchell & Sharp 1964 O Brien 1966) Compounds reacting with sulphydryl groups *e g* sodium *iodoacetate* or p-chloromercuribenzoate (*PCMB*) *inhibit clumping* and swelling of platelets although fairly high concentrations must be used in plasma Ethylenediamine tetracetic acid *(EDTA)* and toluene sulphonyl arginine methyl ester (*TAMe*) inhibit aggregation but not the change in shape and therefore probably act at a later stage (O Brien 1966) *TAMe* is a competitive inhibitor of thrombin) Low concentrations of *PCMB N ethyl* maleimide (NEM) and methylmercuric nitrate (MMN) inhibit aggregation of washed canine platelets by thrombin and ADP (Robinson Mason & Wagner 1963) Although potassium uptake by platelets is not inhibited either by iodoacetate or potassium cyanide platelets incubated with both enzyme inhibitors together cannot take up potassium Platelets can obtain energy for potassium transport either from respiration or glycolysis and both pathways must be blocked to prevent ion transport (Gorstein & Carroll 1964) Weissbach & Redfield (1961) came to the same conclusion about the uptake of 5 HT by platelets Heparin prevents clumping by thrombin only (Cuthbertson & Mills 1963)

The role of platelets in coagulation and haemostasis can be summarized as follows Whenever a blood vessel is injured blood *platelets immediately* begin to adhere to the site of the endothelial lesion (Bounameaux 1957) Within a few seconds ADP and *thrombin are formed* at the site of the vessel injury (Marr Barboriak & Johnson 1965) An early synergistic action between ADP and thrombin *may* be a prominent feature of platelet aggregation and haemostasis The platelets rapidly aggregate undergo viscous metamorphosis and *partly* disintegrate It is generally assumed that, during viscous metamorphosis lipids or

the coagulation of blood began when the platelets aggregated An accelerator of blood coagulation in the platelets was *suggested* already by Hayem (1882), and demonstrated by Delezenne (1897) In 1882 *Bizzozero* noticed that the platelets were involved in some way in intravascular clot formation, and that they disappeared during this process

Several investigators, using a variety of techniques, found that the *aggregation of* platelets preceded even the earliest fibrin formation (Ferguson 1934, *Fonio 1940,* Haydon 1957) Electron microscopical studies have made it possible to examine *the blood* platelets closely *and to follow* their morphological changes, as well as the morphology of the blood *coagulum The* coagulum of blood was found to be preceded by marked changes *in the platelets* (Bloom 1955)

A variety of factors induce platelets to aggregate *in vitro* Contact with a foreign surface as well as number of more or less well defined compounds such as collagen fibres, connective tissue particles, latex particles fibrin thrombin, adenosine diphosphate (ADP) and aggregated γ globulins have been claimed to be capable of initiating platelet aggregation (*cf* Tocantins 1938, Hellem 1960 Marcus & Zucker 1965)

The ADP induced aggregation is a reversible phenomenon, probably due to further degradation of the nucleotide The adenosine monophosphate formed has been shown to antagonize the aggregating effect of ADP The aggregates become unstable and the platelets are released (Born & Cross 1964) Analogues of adenosine (2 chloro adenosine 2 bromo-adenosine and others) also inhibit the aggregation of platelets and may cause vasodilatation in man (Born 1966) The ADP aggregation of platelets probably depends on the presence of a high *molecular* plasma cofactor present in highly purified fibrinogen (Cross 1964)

The mechanism of ADP aggregation remains unsettled Born (1965) suggested *that ADP* bombards specific receptor sites of the platelets, *thus* producing a configurational change which leads to aggregation

It has also been postulated that ADP *inhibits* the splitting of ATP by ATPase This might lead to defects in the cell membrane and exposion of adhesive sites of platelets and permit aggregation (Salzman, Chambers & Neri 1966)

Thrombin induces aggregation of platelets *and also acts synergistically with* ADP (Niewiarowski & Thomas 1966) However *the latter* authors also found that washed platelets were rapidly aggregated by thrombin, *but not by* ADP Thrombin induced aggregation also releases ADP *from the* platelets and it has been *suggested that* this release may be responsible for the aggregating activity of thrombin Thrombin does not however only produce aggregation of platelets but its action also leads to further changes (viscous metamorphosis) (Luscher 1956) According to Born (1966) however the aggregation of platelets by the ADP mechanism was found in several animal species in which it had been investigated Thus it is probable that ADP and thrombin do not have the same site of action on platelets In some *lower* vertebrates however platelet aggregation seems to occur after addition of homologous thrombin but not of ADP (Belamarich *et al* 1966)

It has recently been shown that aggregation is preceded by a change in shape of the platelets from a smooth disc to a spiny

Platelets and heparin

The effects of heparin on platelets have given rise to controversy

According to some authors heparin inhibits the aggregation and disintregration of platelets (Best Cowan & McLean 1938 Solandt & Best 1940 Wright 1941 Baronofsky & Quick 1943 Ollgaard 1943 Moolten *et al* 1949) Other authors have noted a decrease in the number of platelets (Copley & Robbs 1942 Copley & Houlihan 1947 Fidlar & Jaques 1948)

Lundevall (1958) and Hellem (1960) found that small doses of heparin *in vitro* could not prevent platelet clumping even if they were sufficient to prevent coagulation The number of non adhesive platelets however showed a constant increase with rising concentrations of heparin

Concentrations of heparin in excess of 0 1 mg/ml plasma protected the clot retraction function of platelets Such concentrations further prevent agglutination The platelet count was for exemple not altered by rotation in Erlenmeyer flasks (to produce platelet damage) although the cells appeared distorted on phase microscopy Similar results were obtained when platelets suspended in heparinized plasma were subjected to repeated centrifugation and resuspension (Hartmann 1961)

An inverse relation between platelet concentration and heparin activity has been described by several authors Conley, Hartmann & Calley (1949) found the clotting of blood *in vitro* to be inversely proportional to the concentration of platelets present

Platelet factor 4 seems to be a high molecular protein capable of neutralizing heparin and heparin like substances such as dextran sulphate and is probably also the lipoprotein lipase inhibitor of platelets (Deutsch & Kain 1961 Mitchell 1959)

Little (1959) and Bernstock & Hirson (1960) reported successful treatment of thrombotic thrombocytopaenic purpura with small doses af heparin This disorder is characterized by the presence of platelet thrombi in the vessels composed mainly of platelets and fibrin

lipoproteins (platelet factor 3) essential for the generation of thrombin are relased from the platelets (cf Bettex Galland & Luscher 1965, Marcus & Zucker 1965) Fibrin strands appear in connexion with the disintegration of platelets These events result in a firm haemostatic plug, composed mainly of platelets and fibrin

Platelets and 5 hydroxytryptamine (5 HT)

As early as 1886, Ludowig & Schmidt observed that serum and platelet extracts promote vasoconstriction The vasoconstrictive substance is bound to the platelets (Stewart & Zucker 1913, Janeway Richardson & Clark 1918) It acts only in vessels with muscular walls and not on capillaries (Zucker 1947) Zucker also noted that, when a platelet plug was formed in an injured vessel constriction took place in undamaged adjacent vessels The active substance stored in the platelets was 5 hydroxytryptamine (5 HT) (Rapport Green & Page 1948) Details of the identification of 5 HT in various tissues of a number of species, and speculations on its possible physiological role, have been presented in excellent reviews by Page (1954, 1958) Mitchell & Sharp (1964) noted that 5 HT adrenaline and noradrenaline, which normally occur in platelets may cause aggregation Zucker & Borelli (1955) noted that thrombin releases 5 HT from platelets Low concentrations of thrombin or trypsin release 80 per cent of platelet 5 HT (Buckingham & Maynort 1964) When platelets react with connective tissue particles, 5 HT and ADP are also liberated (Spaet & Zucker 1964)

Waalkes *et al* (1957) observed that 5 HT and histamine were released from rabbit platelets during anaphylaxis when an antigen was added to whole blood of sensitized rabbits No increase in the 5 HT content of plasma was found if the rabbits were pretreated with the Rauwolfia alkaloid reserpine They suggested the major portion of the rise in plasma 5 HT to be secondary to a release from disrupted blood platelets A comparable release of 5 HT from platelets occurs during antigen antibody interaction *in vitro* Release of 5 HT and histamine from platelets of rabbits sensitized by antigen has been shown to occur only if plasma is present (Humprey & Jaques 1955) Glycogen, *n* decylamide and phospholipase A also cause a release of 5 HT and histamine from rabbit blood platelets (Waalkes & Coburn 1959 Westerholm 1964) Reserpine tyramine chloropromazine and imipramine release 5 HT but not histamine from the platelets (McLean Nicholson & Hertler 1963) Ninhydrin and allicin inhibit the antigen induced 5 HT release (Westerholm 1964)

Platelets and megakaryocytes cannot synthesize 5 HT, whereas platelets concentrate it by an active transport mechanism Potassium and phosphate ions are required for maximal uptake (Weissbach & Redfield 1961) Inhibitors of oxidative enzymes such as fluoride and 2,4 dinitrophenol have been shown to inhibit 5 HT uptake The data suggest that both glycolysis and respiration may supply energy for 5 HT uptake

Thrombin rapidly liberates 5 HT from the platelets As a result serum contains large amounts of 5 HT whereas plasma does not (Zucker & Borelli 1955)

Heparin inhibits the aggregation of platelets by thrombin, as well as the thrombin induced release of 5 HT from these cells (Gaintner Jackson & Maynert 1962)

when an antigen was added to whole blood of normal rabbits and of sensitized rabbits 6 weeks after the onset of sensitization After addition of diluted horse serum (1 50) or 0 9 per cent saline to whole blood the blood was centrifuged for 20 minutes at 185 g The 5 HT content of plasma of the sensitized rabbits given antigen was several times higher than that after 0 9 per cent saline The control group showed no changes

The release of 5 HT from the blood platelets after addition of antigen *in vitro* strongly indicates that platelets are damaged during antigen antibody reactions

Platelets and heparin

The effect of heparin on the disintegration of platelets during antigen antibody reactions was studied *in vitro* To diluted whole blood (150 000—200 000 platelets/ml) of sensitized rabbits and controls 0 9 per cent saline or antigen was added No change in platelet counts appeared when 0 9 per cent saline was added to the samples A marked decrease occurred when antigen was added to blood of sensitized rabbits A concentration of < 10 i u of heparin/ml was sufficient to prevent the decrease in platelet count (II)

The heparin dose chosen to prevent platelet agglutination was not sufficient to inhibit the release of 5 HT from the platelets during antigen antibody reactions Thus some change in the platelets seems to have occurred judging by the release of 5 HT Large doses of heparin (100—1000 i u /ml) could however prevent these changes (IV)

The circulating platelets are not saturated with 5 HT Incubation of platelets *in vitro* with an excess of 5 HT greatly enhanced the content of the amine (IV)

STUDIES IN VIVO (I—IX)

Antigen Antibody Reaction

Sensitization

Platelets After the sensitization schedule was completed platelet counts were made every second day in rabbits given a daily injection of 1 ml of horse serum i p for 6 days Control rabbits received 1 ml of 0 9 per cent saline instead of horse serum The control group showed no change in platelet count the sensitized group a significant decrease a few days after completed sensitization ($p < 0.01$) Only 90 000 platelets/μl were present in the circulating blood of a rabbit which died on the 15th day with symptoms resembling an anaphylactic reaction (II)

Anaphylactic reaction

Platelets and coagulation factors Blood platelets and plasma coagulation factors were studied both before and at intervals after anaphylactic shock, induced in sensitized rabbits by giving 1 ml of horse serum intra arterially 6 weeks after the onset of sensitization (I and III) All rabbits including controls were anaesthetized The controls received 0 9 per cent saline instead of horse serum.

The controls showed no significant decrease in platelets The fibrinogen content decreased slightly in only 1 of 8 controls probably due to the haemodilution caused by frequent blood sampling An increase in the activity of factors V and IX appeared in some experiments although — for the same reason — a decrease was expected A minor increase in fibrinolytic activity occurred in 3 of the 8 controls

In the anaphylactic rabbits the coagulation time was successively prolonged The

PRESENT INVESTIGATIONS

In 1958, when working with platelet preparations, I observed that heparin decreased the aggregation of washed blood platelets *in vitro* (see p 13)

Since I knew that platelets decrease during an anaphylactic reaction, I tried to analyze the relation between heparin and anaphylactic phenomena

The possibility of a common pathophysiological pathway, involving platelet changes with release of biologically active substances in man, incited me to make a more detailed study of the platelets under different experimental conditions

STUDIES IN VITRO (II and IV)

Antigen-Antibody Reaction

Platelets

Addition of antigen *in vitro* to whole blood of sensitized rabbits resulted in a significant decrease in platelet number ($p < 0.01$) No change in the platelet count was noted when the antigen (horse serum diluted 1 50) was added to blood of normal rabbits, or when 0 9 per cent saline was added to blood of sensitized or normal rabbits The platelet counts were made 30 minutes after addition of the antigen (II) This study confirmed earlier findings of a decrease in platelet count during antigen antibody reactions *in vitro*

Changes in platelets during antigen antibody formation during a sensitization period might influence the coagulation of blood The effect of antigenic horse serum on the coagulation time of rabbit whole blood was therefore determined *in vitro* with the silicone technique The blood was withdrawn through a teflon catheter in the carotid artery on the 14th day of sensitization Control rabbits received 0 9 per cent saline instead of horse serum Different concentrations of horse serum did not alter the coagulation time in the control group *In the sensitized group, the coagulation time* was shortened already when 0 9 per cent saline was added Significant shortening ($p < 0.05$) of the coagulation time appeared with rising concentrations of the antigen (II)

To obtain further evidence of the role of platelet disintegration during antigen antibody reactions *in vitro*, the aforementioned experiments were repeated with plasma enriched by or poor in platelets The clotting time of recalcified platelet enriched plasma of sensitized rabbits was inversely proportional to the antigen concentration added They were significantly *shorter than in platelet deficient plasma* from the same animals ($p < 0.01$) In normal plasma, different amounts of platelets did not affect the coagulation time, nor did different amounts of added antigen

The agglutination of platelets during an antigen antibody reaction thus seems to be responsible for the decrease in coagulation time noted in blood of sensitized rabbits

Platelets and 5 hydroxytryptamine (5 HT)

Almost all 5 hydroxytryptamine in the blood is bound to the platelets The release of 5 HT from platelets was studied *in vitro*

when an antigen was added to whole blood of normal rabbits and of sensitized rabbits 6 weeks after the onset of sensitization After addition of diluted horse serum (1 50) or 0 9 per cent saline to whole blood the blood was centrifuged for 20 minutes at 185 *g* The 5 HT content of plasma of the sensitized rabbits given antigen was several *times higher than that after 0 9 per cent* saline The control group showed no changes

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In the anaphylactic rabbits the coagulation time was successively *prolonged The*

platelets decreased already a few minutes after the onset of anaphylactic shock. Factor IX (haemophilia B), factor V (proaccelerin), and factors II and VII (prothrombin-proconvertin) fell continuously for about 3 hours, starting later than the platelets. The fibrinogen was not decreased until 2 hours after the onset of shock.

During anaphylaxis, the plasma fibrinolytic activity increased slightly in 3 rabbits. Simultaneously with these changes in the coagulation factors, the antithrombic activity rose within 2 hours of the shock dose, with a maximum in the first hour. Thrombocytosis (values up to 850,000) occurred within 5 days after the shock.

Platelets and 5-hydroxytryptamine. The concentration of 5-HT in whole blood, plasma and tissues was determined spectrofluorophotometrically before and at intervals after an anaphylactic shock elicited 5 weeks after the onset of sensitization (when the antigen-antibody titre was expected to be high), by giving 1 ml of diluted horse serum i.v. to sensitized anaesthetized rabbits (IV).

The 5-HT content of whole blood decreased from an average 4.0 to 1.4 μg/ml within 3 minutes of the shock dose. In platelet-free plasma, however, there was a marked increase in the 5-HT concentration within 2 minutes of the shock; it was no longer present 10 minutes later.

A similar increase in 5-HT was found in the lungs, where it was noted 10 minutes after the onset of shock but not 50 minutes later.

The fate of the 5-HT released from the platelets was indicated by the urinary 5-HT metabolite 5-HIAA, which was twice as high after the shock as before (IV).

The low 5-HT content of whole blood persisted for about 24 hours. The platelet count, on the contrary, regained the normal level within 2—3 hours. This evidently indicates a rapid release of 5-HT-free platelets from the marrow, replacing the destroyed ones.

Most of the 5-HT in the body is stored in the intestines. A release of the amine from this tissue is difficult to detect, because of the wide normal range of variation. Consequently, the implications of the unchanged 5-HT values in the upper 10 cm of the gut after the shock dose cannot be evaluated.

Heparin Effects on Platelets, Coagulation Factors and 5-HT in Sensitization and during Anaphylaxis

Sensitization

Platelets. After completion of the sensitization schedule, platelet counts were performed every second day in sensitized animals, given a daily injection of 10 mg of heparin into an ear vein. No change in platelet count was observed.

Thus, heparin was able to prevent the decrease in platelet count which occurs in rabbits during the sensitization period (p. 15).

Anaphylactic reaction

a. *Platelets.* Platelet counts were made in 2 groups of sensitized rabbits before and after injection of horse serum on two different occasions. Ten rabbits were given 1 ml of horse serum 2 weeks after the onset of sensitization. Five were pretreated with 10 mg of heparin 30 minutes before the antigen injection and another 5 received 1 mg. The number of circulating platelets fell in the untreated rabbits and in those treated with 1 mg of heparin. No change

in platelet count occurred in the group given 10 mg (II) The same experiment was repeated with 10 rabbits 6 weeks after the onset of sensitization when the antibody titre was expected to be high Diluted horse serum (1 50 in 0 9 per cent saline) was then used as antigen Five animals were given 100 mg of heparin 20 minutes before the antigen injection In the untreated group a marked decrease in the number of circulating platelets appeared within 10 minutes of the antigen injection ($p < 0.005$) and persisted for more than 1 hour In the heparin treated group no change in platelet count occurred after the i v injection of antigen (IV) (The high dose of heparin was used because studies *in vitro* showed such doses to be necessary to prevent the release of 5 HT from platelets)

Thus heparin may prevent the fall of circulating platelets after injection of antigen

b *Platelets and coagulation factors* The clotting of blood is usually described as occurring in three main stages

1 Formation of plasma thromboplastin in which the platelets antihaemophilic factors A and B (VIII and IX) and calcium ions participate
2 Formation of thrombin from prothrombin under the influence of plasma or tissue thromboplastin factors V and X and calcium ions In addition, factor VII is needed in a system where tissue thromboplastin takes part in the reaction
3 Transformation of fibrinogen into fibrin under the influence of the proteolytic enzyme thrombin

In addition to the studies of the platelets some of these stages of blood coagulation were surveyed

Platelet counts prothrombin proconvertin activity and fibrinogen level were determined in rabbits on the 14th and 15th day after the onset of sensitization, before injection of the antigen as well as 1, 2 and 4 hours after it A significant decrease in platelet counts and prothrombin proconvertin activity was found on the 14th day On the 15th day the values had regained normal levels The fibrinogen content had then increased markedly when compared to that on the previous day After a second dose of antigen a diminution of platelet counts and prothrombin proconvertin activity was again noted within 1 hour A decrease in the fibrinogen content was observed 4 hours after the antigen injection Animals pretreated with 10 mg of heparin 30 minutes before the antigen dose showed no changes in platelet count prothrombin proconvertin activity and fibrinogen Heparin thus inhibits the consumption of factors inherent in the three stages of clotting

c *5 hydroxytryptamine* The effect of heparin in the release of 5 HT after eliciting an anaphylactic reaction was studied in sensitized rabbits 5 weeks after the onset of sensitization Sensitized animals given 0 9 per cent saline instead of heparin served as controls

I v injection of a antigen (1 ml of diluted horse serum) decreased the whole blood content of 5 HT but increased the 5 HT of the plasma and lung tissue as well as the 5 HIAA content of the urine Heparin treated animals showed no changes

Burn Injuries

The apparent consumption of platelets and coagulation factors observed during anaphylaxis (I—III) is similar to that noted

platelets decreased already a few minutes after the onset of anaphylactic shock Factor IX (haemophilia B), factor V (proaccelerin), and factors II and VII (prothrombin proconvertin) fell continuously for about 3 hours, starting later than the platelets The fibrinogen was not decreased until 2 hours after the onset of shock

During anaphylaxis, the plasma fibrinolytic activity increased slightly in 3 rabbits Simultaneously with these changes in the coagulation factors, the antithrombic activity rose within 2 hours of the shock dose, with a maximum in the first hour Thrombocytosis (values up to 850,000) occurred within 5 days after the shock

Platelets and 5 hydroxytryptamine The concentration of 5 HT in whole blood, plasma and tissues was determined spectrofluorophotometrically before and at intervals after an anaphylactic shock, elicited 5 weeks after the onset of sensitization (when the antigen antibody titre was expected to be high), by giving 1 ml of diluted horse serum i v to sensitized anaesthetized rabbits (IV)

The 5 HT content of whole blood decreased from an average 4 0 to 1 4 μg/ml within 3 minutes of the shock dose In platelet free plasma however there was a marked increase in the 5 HT concentration within 2 minutes of the shock, it was no longer present 10 minutes later

A similar increase in 5 HT was found in the lungs where it was noted 10 minutes after the onset of shock but not 50 minutes later

The fate of the 5 HT released from the platelets was indicated by the urinary 5 HT metabolite 5 HIAA which was twice as high after the shock as before (IV)

The low 5 HT content of whole blood persisted for about 24 hours The platelet count, on the contrary, regained the normal level within 2—3 hours This evidently indicates a rapid release of 5 HT free platelets from the marrow, replacing the destroyed ones

Most of the 5 HT in the body is stored in the intestines A release of the amine from this tissue is difficult to detect, because of the wide normal range of variation Consequently, the implications of the unchanged 5 HT values in the upper 10 cm of the gut after the shock dose cannot be evaluated

Heparin Effects on Platelets, Coagulation Factors and 5-HT in Sensitization and during Anaphylaxis

Sensitization

Platelets After completion of the sensitization schedule, platelet counts were performed every second day in sensitized animals, given a daily injection of 10 mg of heparin into an ear vein No change in platelet count was observed

Thus heparin was able to prevent the decrease in platelet count which occurs in rabbits during the sensitization period (p 15)

Anaphylactic reaction

a *Platelets* Platelet counts were made in 2 groups of sensitized rabbits before and after injection of horse serum on two different occasions Ten rabbits were given 1 ml of horse serum 2 weeks after the onset of sensitization Five were pretreated with 10 mg of heparin 30 minutes before the antigen injection and another 5 received 1 mg The number of circulating platelets fell in the untreated rabbits and in those treated with 1 mg of heparin No change

Table 1

Laboratory findings

Date	Hemoglobin	White blood cells	Platelets	Fibrinogen g per 100 ml	Thrombo test per cent	Coagulation time in plastic tubes
22 11	13 9	3 600	69 000			
23 11	13 8	2 500	90 000	0 21	23	12 15
24 11	13 7	2 600	127 000	0 13	26	11 00"
25 11	13 0	2 900	147 000	0 26	58	14 15"
28 11	11 6	3 200	249 000	0 31		16 30"
1 12	12 7		322 000			
[illegible] 12	13 4	5 800	445 000			
Normal values	13 5—18 5	3000—8000	150 000—350 000	0 26±	70—100	14—30 min

urinary excretion of 5 HIAA was considerably increased in 4 of the 5 patients with severe burns The highest value measured was 32 5 mg/24 hours in one patient The mean value in 30 students of both sexes was 4 8 ± 2 4 mg/24 hours (VII)

Clinical Studies on Hypersensitivity

During my work with experimental animals in anaphylaxis and burn injuries I studied the platelets and coagulation factors in patients with spontaneous or induced hypersensitivity reactions

Acute episode of hypersensitivity

Case 1 M A a 63 year old man was vaccinated against variola on Nov 7 1966 Nine days later he felt ill his temperature rose to 39° C, and generalized haemorrhagic purpura with proteinuria appeared during the next 6 days He was hospitalized on Nov 22 No manifestations of shock were present Fever and purpura regressed without treatment within 3 and 10 days of admission respectively The laboratory findings are shown in table 1 No signs of haemolysis or liver damage were observed

Coagulation studies Low platelet count and low coagulation activity as measured in thrombotest and somewhat shortened coagulation time in plastic tubes were noted during the first 3 days The fibrinogen concentration was normal during the first 2 days but fell on the 3rd day in hospital A continuous rise in platelet count was observed for 2 weeks the thrombotest and fibrinogen levels were normalized

Comment The coagulation findings indicate a consumption of coagulation factors during acute generalized purpura caused by vaccination against variola

Heparin treatment of provoked hypersensitivity

Ten persons hypersensitive to at least one allergen were tested with standard extracts of the allergen The dose chosen was large enough to provoke temporary thrombocytopoenia and produced a distinct local reac

after injection of thromboplastic material, e g induced intravascular coagulation (cf Quick *et al* 1959) It was therefore of interest to study another trauma, the burn injury, when such substances may be released from burned areas, and to ascertain whether a similar pattern of reactions occurred

Platelets

A decrease in the number of circulating platelets (average from 313,000 to 186,000/μl) was noted within 2 hours of the burn injury in 15 untreated rabbits Altogether 13 were dead within 10 hours (20 per cent third-degree burn produced with 65° C water for 2 minutes) The platelets returned to the normal level within 6 hours (VII, VIII, IX) A continuous increase in the number of platelets was recorded in 2 rabbits which survived for more than 2 days Values up to 800 000 platelets/μl were noted

Coagulation factors

The coagulation time was prolonged in 5 unheparinized rabbits within 30 minutes of the burn injury, and this prolongation persisted in the 4 which died within 7 hours of the injury

The prothrombin proconvertin activity decreased in 5 rabbits within one hour of the burn injury The fibrinogen content of blood showed no constant variations except that the animals which died during the experiment showed a drop in the level at the last determination before death (IX) The antithrombic titre was greatly increased during the first hours after the burn injury, and corresponded to between 1 and 2 i u of heparin/ml of whole blood

5 Hydroxytryptamine

The platelet count and 5 HT content of blood were decreased during the first hours after the burn injury in 5 rabbits The platelets returned to the normal level within 6 hours, whereas the decrease in 5 HT concentration in blood persisted for at least 12 hours in the animals which survived Thus, at this moment, the 5 HT content of the platelets was low During the first 3 hours, however, the 5 HT content per platelet seemed to be increased This is probably to be ascribed to the fact that cells are not saturated with the amine, since incubation of platelets *in vitro* with an excess of 5 HT greatly enhanced their ability to absorb 5 HT added to plasma (VII) As under normal conditions, platelet deficient plasma of burn injured animals contained no detectable amounts of 5 HT An increase in the 5 HT content of lung tissue was noted 90 minutes after the burn No variation was noted in the 5 HT concentration in the intestines

Effect of heparin in burn injured rabbits

Heparin pretreatment prolonged the survival time of the animals and prevented the decrease in platelet count in prothrombin proconvertin activity, fibrinogen concentration and concentration of 5 HT in blood, as well as the increase of the amine in lung tissue (VIII—IX)

Clinical studies of burn injuries

Determinations of 5 HT in blisters and 5 HIAA in urine (24 hour samples) were made in 5 patients with severe burns Most of the blisters contained small amounts of 5 HT (about 0 1 μg/ml blister fluid) Not even traces of 5 HT could be detected in the abdominal skin of 6 controls The

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GENERAL DISCUSSION

The origin of platelets from the megakaryocytes (Wright 1910) is now generally accepted They are essential for the normal arrest of bleeding and have a complex role in the haemostatic mechanism Little is however known of the mechanism governing the number of circulating platelets Their rate of production release from the bone marrow and removal from the circulation are unsettled questions

The platelets have been shown to decrease gradually in size as they age in the circulation (McDonald, Odell & Gosslee 1964) A random destruction of platelets mediated by mechanisms related to blood *coagulation is thought to play a major role* in terminating their life Their survival time is increased by heparin and by vegetable fat diet It is recreased by hypercoagulability (Adelson *et al* 1963 Mustard *et al* 1964) Atherosclerotic patients and those with recurrent thrombophlebitis and thromboembolism have a shortened platelet survival time (Mustard *et al* 1964)

Thrombocytopoenia probably due to diminished platelet survival is found in immune mechanisms (*e g* drug hypersensitivity food allergy idiopathic thrombocytopenic purpura autoimmune haemolytic anaemia disseminated lupus erythematosus neonatal thrombocytopoenia Aldrich s syndrome and hyperimmune states following acute infection and malignant diseases)

In allergic reactions a decrease in the number of circulating platelets is common Nilzen (1953) elaborated this phenomenon into a useful clinical test

Platelet adhesion aggregation and viscous metamorphosis are thus to be expected not only when haemostasis is needed but also in a great variety of pathophysiological phenomena

Platelets appear to have further important functions not yet clarified The work of Bloom, Gustavson & Swensson (1955) suggests that the platelets are able to take up quartz particles Danon Jerushalmy & de Vries (1959) showed that virus particles are incorporated into the platelets These observations are in accordance with the suggestion made by Tocantins (1938) *i e* that platelets may be of importance in body *defence against foreign particles and micro* organisms If and to what extent inclusion of foreign particles may cause aggregation and destruction of platelets has not yet been determined

During antigen antibody reaction, the platelets agglutinate and disintegrate (Rocha e Silva 1950 Miescher & Straessle 1956) They release a lipid or lipoprotein, platelet factor 3 which is involved in the enzymic plasma reactions leading to prothrombin activation and biologically active amines (5 HT histamine adrenaline) It is therefore of importance to establish the role of platelet disintegration caused by various agents and traumas

The main part of the present investigation deals with the disappearance of platelets during experimental anaphylaxis and burn injuries A decrease in platelet count and in the level of coagulation factors was accompanied by a release of 5 HT (I—IV

tion Platelet counts were made immediately before and at intervals after intradermal injection of the specific allergen The test was repeated 2 weeks later The patients received 100 or 200 mg of heparin or 0 9 per cent saline i v 30 minutes before the test Platelet counts were made immediately before the heparin or saline injection

All patients given 0 9 per cent saline showed a significant fall in the number of circulating platelets from an average 230,000 to 129,000/μl within 20 minutes ($p < 0.05$) A pronounced general reaction with rhinitis, cough, bronchial asthma and faintness developed in one patient Isoprenalin spray administered 55 minutes after the injection of allergen promptly relieved most of the symptoms Some hours later, an urticarial rash appeared and persisted together with conjunctivitis and fatigue during the following day When the test was repeated after heparin treatment, the platelet count remained unchanged, and no local or generalized symptoms or signs appeared

Heparin Treatment of Biliary Cirrhosis with Bleeding

After my observations of the heparin effect during anaphylaxis in rabbits, I had an opportunity, in 1960 of studying a woman with a similar coagulation picture She suffered from biliary cirrhosis of the liver with continuous melaera, periods of acute bleeding and manifestations of shock but without evidence of anaphylaxis

Coagulation analysis showed a shortened coagulation time in siliconized tubes, lowered platelet counts, and low levels of prothrombin proconvertin activity, factors V, IX and fibrinogen No fibrinolysis or circulating anticoagulants were present An increase in AHF activity (factor VIII) was observed During clinical manifestations of shock, a rapid fall was noted in the levels of the aforementioned coagulation factors (V) During an episode of bleeding and shock, her fibrinogen level fell within one hour from 0 34 to 0 19 g/100 ml of blood It was observed that during periods of low clotting time with low platelet counts and low prothrombin proconvertin activity (less than 2 per cent) and fibrinogen the patient felt fairly well, despite her haemoglobin being as low as 6 g/100 ml Whole blood transfusions (blood freshly drawn from the donors with the silicone technique) resulted in rigors vomiting of blood, melaena and shock Washed blood cells had no adverse effect

On two occasions, she was treated with heparin (500 mg/24 hours) during acute bleeding and shock Both times the shock was overcome within 2 hours During continuous heparin therapy, he intestinal bleeding ceased The haemoglobin level rose as well as the platelet count fibrinogen content and prothrombin proconvertin activity Moreover, she tolerated whole blood therapy (V)

Heparin treatment of similar bleeding episodes has subsequently been reported by von Franken *et al* (1963)

importance for the preservation of a native conformation of fibrinogen molecules (Blombäck *et al* 1966)

Heparin prevented most of the proteinuria and hematuria which otherwise occurs in some rabbits already during sensitization and after the anaphylactic shock (Johansson 1967) This finding might be of importance for understanding of the reactions leading to renal injuries after trauma toxins and inflammation

Pretreatment with heparin has a protective action against shock and consumption of coagulation factors in certain bleeding states in humans *i e* obstetric shock with release of large amounts of thromboplastic substances (Schneider 1959 Pfau Lasch & Gunther 1960) bleeding states in liver cirrhosis (Johansson 1960 VI von Francken *et al* 1963) and purpura fulminans (Hjort *et al* 1964) The protective action of heparin has also been demonstrated in endotoxin shock in animals (Good & Thomas 1953) anaphylaxis (Johansson 1960 1961 1964 I—IV Dhar & Sanyal 1962) and burn injuries (Johansson 1961 1964 VIII IX)

It seems reasonable to suggest that heparin therapy should be tried in all cases in which platelet damage occurs or in which a shortened survival time of platelets is suspected (*cf* p 21)

The fact that antithrombic substances are formed during coagulation of blood (Nilsson & Wenkert 1954) and that an antithrombic substance probably heparin is released during anaphylaxis indicate that such substances may be of importance for prevention of intravascular coagulation One rabbit in Paper III (no 13) released antithrombic substance corresponding to 5 i u of heparin/ml of blood 60 minutes after the shock dose This corresponds roughly to 20 000 i u. (200 mg) of heparin in man, a sufficient therapeutic dose It is interesting to note that although the antithrombic level was exceptionally high, the coagulation time decreased to normal, and rising levels of coagulation factors were observed This animal survived This would indicate that the antithrombic substance released is not heparin or that heparin is inactivated by some kinases (*e g* thromboplastin released from tissues or formed during the clotting process) Large doses of heparin is needed to prolong the coagulation time of blood if its reactivity is lowerred due to decreased level of heparin co factor (Olsson 1963) or for direct enzymatic inhibition by its strong electric charge (Jorpes 1946) The fate of heparin co-factor during anaphylaxis is unsettled

Heparin prevents the formation of plasma thromboplastin in as low concentrations as 1/20—1/30 i u /ml About 10 times this dose is required to neutralize formed plasma thromboplastin (MacMillan & Brown 1954) Much larger doses of heparin are necessary to prevent prothrombin conversion to thrombin (Ferguson 1937 Brinkhous *et al* 1939)

During anaphylaxis and burn injuries 5 HT is released from the platelets An increase in the amine was found in lung tissue (IV VII) where disintegrated platelets are accumulated and are lysed after antigen antibody reactions (Rocha e Silva 1950) An increased amount of the metabolite 5 HIAA in urine was also noted Heparin treatment prevents these events (IV VII—VIII) as does EDTA (2 mg/ml Johansson unpublished observation 1964) The concentrations of 5 HT found in blood and tissues are in good accordance with those reported by Waalkes *et al* 1957

VII—IX) These laboratory findings are similar to those observed in induced intravascular coagulation (Schneider 1951, Quick *et al* 1959) and indicate the existence of intravascular coagulation The definite proof of such coagulation would be the demonstration of fibrinopeptides in blood Such work has not yet been successful Similar features indicative of intravascular coagulation were observed clinically (biliary cirrhosis with bleeding tendency and shock, and generalized haemorrhagic purpura during vaccinia)

The fact that, under the given experimental conditions, heparin prevented the decrease in platelet count, coagulation factors and release of 5 HT and, in addition, relieved a variety of symptoms — including haemorrhage and shock — provides evidence in favour of the idea of intravascular coagulation (Johansson 1960, VI and p 19) Several reports of consumption of coagulation factors can be interpreted in the same way *i e*, in obstetric shock (Schneider 1951 1959), giant haemangioma (Blix & Aas 1961), thrombotic thrombocytopoenic purpura (Ruffolo, Pease & Cooper 1962), acute pancreatitis (Shinowara *et al* 1963), purpura fulminans (Hjort *et al* 1964), fat embolism (Thomas 1954), Sanarelli Schwartzman reaction (Good & Thomas 1953 Pfau Lasch & Gunther 1960) Cases of possible intravascular coagulation have been reviewed by McKay (1965)

The following mechanisms could be envisaged as promoting intravascular coagulation due to anaphylaxis The antigen antibody reaction leads to 1 Platelet damage by an antigen antibody reaction, with disintegration and lysis of these calls Platelet factor 3 is released and activates the intrinsic coagulation system 2 Injury to the vascular endothelium, followed by a release of thromboplastic material activating the extrinsic clotting system Manifest thrombosis is, however, an exceptional outcome even if the coagulation system is activated This may be due to the action of the fibrinolytic system and the reticuloendothelial system (RES) The latter is capable of preventing widespread intravascular fibrin deposition by phagocytosis of small fibrin aggregates (Lee 1962) Many cases have, however been described in which the mechanisms preventing recurrent thrombosis have been inadequate (*e g* McKay 1965) In one interesting case, Nilsson *et al* (1961) could demonstrate insufficient fibrinolysis to be the cause

As early as 1927, Kyes & Strauser found that heparin protected animals against anaphylaxis when given before the eliciting shock dose This finding could not be confirmed by Reed & Lamson (1927), nor by Reed (1930) and others (*cf* p 8) Since then, no report on this topic was published until the appearance of Paper IV (Johansson 1960) It was then shown that heparin prevented the decrease in platelet count and the subsequent release of the biologically active amine, 5 hydroxytryptamine, from these cells when given before the shock dose It also prevented the fall in prothrombin proconvertin activity and in the fibrinogen level (I and II) The same effects are achieved by means of disodium ethylene diamine tetraacetic acid (EDTA), if given in concentrations of about 2 mg/ml of blood 20 minutes before the anaphylactic shock dose (Johansson unpublished observation 1964) This dose is sufficient to prevent clotting of blood by removing ionized calcium Ca ions are necessary for platelet aggregation viscous metamorphosis and different stages of blood coagulation Calcium ions may also be of

importance for the preservation of a native conformation of fibrinogen molecules (Blombäck et al 1966)

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I found that after anaphylaxis and burn injuries, the platelet count returned to a normal level earlier than the 5 HT content of blood (almost all 5 HT in blood is bound to the platelets) Evidently, new platelets lacking 5 HT are rapidly mobilized from the bone marrow Thus, the 5 HT of blood is taken up by the platelets during their circulation in the blood An example of the uptake is given in Paper VII

Healthy subjects do not show broncho-constriction after i v administration of aerosol of 5 HT Asthmatic patients, how ever, on the other hand, seem to be more sensitive to the bronchial effect of 5 HT (Michelson & Hollander 1956 Hajos 1962) Patients with a carcinoid tumour often have manifestations resembling those of allergic reactions Thus Thorson (1958) found that 5 of 12 patients (42 per cent) with carcinoid disease had asthma It has been indicated that the therapeutic effect of the butanolamide of 1 methyl lysergic acid in asthmatic and allergic patients is due to its specific inhibition of 5 HT (Ballestro & Zmud 1961, Girard 1961, Hajos 1962)

Pulmonary symptoms of asthmatic type are also seen in other conditions with a decreased platelet count, such as allergic reactions, lupus erythematosus and periarteritis nodosa, and may have the same mechanism

The fall of platelets found in allergic conditions prompted me to investigate the effect of heparin in clinical cases of thrombocytopoenia

In 10 cases of hypersensitivity, heparin prevented a platelet decrease and inhibited the allergic manifestations usually occurring after provocation (V) These results have been confirmed in asthma by Dahl (1962), Hartman (1963) and in asthma, hay fever and weeping eczema by Dougherty & Dolovertz (1964)

After platelet disturbances due to various causes (antigen antibody reactions and burn injuries in experimental animals allergic reactions and bleeding syndromes in man) transient secondary thrombocytosis was noted

Thrombocytosis is in addition, often a sequele of major operations and parturition (Wright 1942), probably due to a compensatory release of newly formed platelets from the marrow Since young platelets seem to be stickier than older ones it is not surprising that Wright found a correlation between elevated platelet count and thrombosis in these conditions

SUMMARY

1 Sensitization of rabbits with horse serum results in a decrease of platelet count and coagulation time of whole blood in siliconized tubes The latter is inversely related to antigen concentration The clotting time of recalcified platelet enriched plasma behaves similarily

2 Anaphylaxis in rabbits sensitized with horse serum leads to

A marked decrease of the platelet count

A decrease of several plasmatic coagulation factors IX V II + VII and fibrinogen

An increase in the antithrombic activity of blood

A release of 5 hydroxytryptamin (5 HT) Increased amounts hereof are found in the lungs The metabolite 5 hydroxyindoleacetic acid (5 HIAA) increases in the urine

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4 5 HIAA increased in the urine after burn injuries in man

5 Similar changes of the coagulation factors as found during anaphylaxis appeared in one case of generalized hemorrhagic purpura due to vaccinia and one patient with biliary cirrhosis

6 Thrombocytosis is noted after anaphylaxis and burn injuries in experimental animals as well as in coagulation syndromes in man

7 Heparin milders the pathophysiological events determined above

8 Heparin treatment increases the survival time of rabbits exposed to trauma by anaphylaxis and burn injuries Clinically it may prevent the symptomes and signs of provoked allergic reactions and may stop bleeding and alley shock in liver cirrhosis

Conclusion

The results of the present investigations indicate that in sensitization anaphylaxis and burn injuries as well as in clinical conditions such as hypersensitive and bleeding states disintegration of platelets elicit or partake in an unspecific reaction leading to a release of 5 HT and consumption of coagulation factors The disintegration of platelets may thus be the trigger of a common pathophysiological reaction intravascular coagulation

This phenomenon seems to have import for divers symptoms and signs in clinical conditions

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8 Heparin treatment increases the survival time of rabbits exposed to trauma by anaphylaxis and burn injuries Clinically it may prevent the symptomes and signs of provoked allergic reactions and may s op bleeding and alley shock in liver cirrhosis

Conclusion

The results of the present investigations indicate that in sensitization anaphylaxis and burn injuries as well as in clinical conditions such as hypersensitive and bleeding states disintegration of platelets elicit or partake in an unspecific reaction leading to a release of 5 HT and consumption of *coagulation factors* The disintegration of platelets may thus be the trigger of a common pathophysiological reaction intra vascular coagulation

This phenomenon seems to have import for divers symptoms and signs in clinical conditions

I found that after anaphylaxis and burn injuries, the platelet count returned to a normal level earlier than the 5 HT content of blood (almost all 5 HT in blood is bound to the platelets) Evidently, new platelets lacking 5 HT are rapidly mobilized from the bone marrow Thus, the 5 HT of blood is taken up by the platelets during their circulation in the blood An example of the uptake is given in Paper VII

Healthy subjects do not show broncho constriction after i v administration of aerosol of 5 HT Asthmatic patients, how ever on the other hand, seem to be more sensitive to the bronchial effect of 5 HT (Michelson & Hollander 1956 Hajos 1962) Patients with a carcinoid tumour often have manifestations resembling those of allergic reactions Thus, Thorson (1958) found that 5 of 12 patients (42 per cent) with carcinoid disease had asthma It has been indicated that the therapeutic effect of the butanolamide of 1 methyl lysergic acid in asthmatic and allergic patients is due to its specific inhibition of 5 HT (Ballestro & Zmud 1961 Girard 1961 Hajos 1962)

Pulmonary symptoms of asthmatic type are also seen in other conditions with a decreased platelet count such as allergic reactions lupus erythematosus and periarteritis nodosa, and may have the same mechanism

The fall of platelets found in allergic conditions prompted me to investigate the effect of heparin in clinical cases of thrombocytopoenia

In 10 cases of hypersensitivity, heparin prevented a platelet decrease and inhibited the allergic manifestations usually occurring after provocation (V) These results have been confirmed in asthma by Dahl (1962), Hartman (1963) and in asthma hay fever and weeping eczema by Dougherty & Dolovertz (1964)

After platelet disturbances due to various causes (antigen antibody reactions and burn injuries in experimental animals, allergic reactions and bleeding syndromes in man) transient secondary thrombocytosis was noted

Thrombocytosis is, in addition, often a sequele of major operations and parturition (Wright 1942), probably due to a compensatory release of newly formed platelets from the marrow Since young platelets seem to be stickier than older ones it is not surprising that Wright found a correlation between elevated platelet count and thrombosis in these conditions

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on the occasion of his sixtieth birthday
March 11, 1967

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TORGNY SJOSTRAND

When *Torgny Sjostrand* reaches the age of 60 years on the eleventh of March this year he can look back with great satisfaction upon a rich contribution to Swedish and Scandinavian medicine, and especially in clinical physiology, where he has been and still is one of the foremost creative and leading forces Torgny Sjostrand s extensive scientific writings began in 1930 with a series of papers on blood distribution and circulation in the lungs and atelektasis which led to a thesis in 1935 The blood supply and distribution of blood to the lungs continued to interest him for many years After studies in England, U S A and Denmark in 1937—38, he returned to the Pharmacological Institute which had become a rallying point for young physiologists under the leadership of Göran Liljestrand. Here the writer of this address had the opportunity of a stimulating collaboration with Sjostrand in studies on kidney extracts and renin discovered by Tigerstedt as well as on pressor substances in urine and plasma

During the second world war, Sjostrand became interested in the poisonous effects of producer gas which led to the famous carbon monoxide method (1948) of determining the amount of blood in the body Prior to this he had also described a method for determining the COHb concentration in the blood through CO analysis of the alveolar air

Already at this time Sjöstrand was very interested in the development of Clinical Physiology, and towards the end of 1940 he began in cooperation with clinical colleagues the study of haemoglobin amount heart volume, and physical working capacity This work attracted much appreciation on an international scale and consolidated his position as one of the leadings forces in this important field Thus he became Associate Professor at the Medical Physiological Central Laboratory at the Karolinska Hospital, followed by an Associate Professorship in Clinical Physiology in 1949 and a full Professorship in this subject in 1956

His interest and achievements in sport physiology led to a chairmanship on the Committee for Health Control of Athletes under the Swedish Athletic Association in 1956 and the Military Medical Examination Centre in 1957 as well as membership on the Board of the State Work Clinic (Statens arbetsklinik) since 1959 In these capacities Sjostrand s work has been extremely beneficial not least for young people s physical training

In 1950 Sjostrand continued his studies on carbon monoxide and in 1951 he could show that carbon monoxide was produced endogenously in the body in the breaking down of haemoglobin a remarkable discovery Here as in other fields, Sjostrand s research work is distinguished by originality and a new grasp of the problem



Torgny Sjostrand

From the University Hospital Uppsala, Sweden (Departments of Clinical Physiology, Diagnostic Radiology and Thoracic Surgery)

Physical Work Capacity, Ecg Reaction to Work Test and Coronary Angiogram in Coronary Artery Disease

By

Nils Holger Areskog, Lars Björk, Viking Olov Björk, Adar Hallén and Gunnar Ström

In clinical medicine, coronary artery disease (C.A.D.) has mainly been diagnosed indirectly from the symptom of angina pectoris and from the evidence of an episode of myocardial infarction. From certain points of view, this qualitative diagnosis needs complementing. Thus while false positive diagnoses are relatively unusual, false negative diagnoses are not uncommon, mostly at a relatively early stage of the disease but often also in advanced cases. Furthermore the prognosis is usually very imprecise in the individual case and the disease and its course cannot be evaluated topically and quantitatively as is needed e.g. in the selection of patients for surgical therapy or in the choice of non surgical therapy. More recently laboratory methods with a bearing on the diagnosis of C.A.D. have been added to clinical medicine, especially the work test with electrocardiography and coronary angiography but also methods for studying left ventricular function such as cineangiography and measurements of cardiac metabolism and pressures.

It is the hope that such methods may eventually provide a much needed complement to the clinical qualitative diagnosis so that early diagnosis may be made in cases without angina pectoris, quantitative and topical evaluation of C.A.D. may become possible, and the prognosis as to infarction morbidity, left ventricular failure and mortality may become more precise.

At present the value of these laboratory methods is under analysis in the literature. The present report is related to part of the general problem and gives mainly the results of work tests and coronary angiography in a material of patients with relatively advanced C.A.D. The conclusions will e.g. concern the occurrence of false negative Ecg and pain reactions to a standardized work test in C.A.D. and the significance of physical work capacity (exercise tolerance) in relation to coronary angiographic changes.

Patient material

The case material consists of 100 male and 11 female patients, examined at the

Many of his articles and essays discuss general problems in clinical research, the physiology of the aged, athlete's heart and training of physio therapists, in the latter sphere his achievements have had great influence

Sjostrand's organising interest and ability have been put to many uses He is chairman of the University Hospital's Chief Physicians Association and deputy member on the board of the Karolinska Hospital In 1966 he was given the respon sible position of Dean of the Medical Faculty at the Karolinska Institute, where his firm resolution and wise deliberations have already procured the respect and affection of his colleagues

As teacher, instructor and adviser of young clinical physiologists he has rendered exceptionally valuable service His wide knowledge is also a sound basis for his important textbook, 'Clinical Physiology", which will be published in time for his 60th birthday

Torgny Sjostrand has, as his friends and colleagues know, a strong cultural and artistic vein to which his beautiful art collection bears witness

Many appreciative and grateful thoughts go to Torgny Sjostrand on his 60th birthday, with heartfelt wishes for continued strength to carry on the work which he has so successfully pursued for many years These wishes are shared by all Scan dinavian physiologists and other colleagues in the preclinical and clinical field

Ulf von Euler

From the University Hospital Uppsala Sweden (Departments of Clinical Physiology Diagnostic Radiology and Thoracic Surgery)

Physical Work Capacity, Ecg Reaction to Work Test and Coronary Angiogram in Coronary Artery Disease

By

Nils Holger Areskog Lars Björk, Viking Olov Björk, Adar Hallen and Gunnar Ström

In clinical medicine, coronary artery disease (C A D) has mainly been diagnosed indirectly from the symptom of angina pectoris, and from the evidence of an episode of myocardial infarction From certain points of view this qualitative diagnosis needs complementing Thus while false positive diagnoses are relatively unusual false negative diagnoses are not uncommon mostly at a relatively early stage of the disease but often also in advanced cases Furthermore the prognosis is usually very unprecise in the individual case and the disease and its course cannot be evaluated topically and quantitatively as is needed e g in the selection of patients for surgical therapy or in the choice of non surgical therapy More recently laboratory methods with a bearing on the diagnosis of C A D have been added to clinical medicine especially the work test with electrocardiography and coronary angiography but also methods for studying *left ventricular function* such as cineangiography and measurements of cardiac metabolism and pressures.

It is the hope that such methods may eventually provide a much needed complement to the clinical qualitative diagnosis so that early diagnosis may be made in cases without angina pectoris, quantitative and topical evaluation of C A D may become possible, and the prognosis as to infarction morbidity left ventricular failure and mortality may become more precise

At present the value of these laboratory methods is under analysis in the literature The present report is related to part of the general problem and gives mainly the results of work tests and coronary angiography in a material of patients with relatively advanced C A D The conclusions will e g concern the occurrence of false negative Ecg and pain reactions to a standardized work test in C A.D and the significance of physical work capacity (exercise tolerance) in relation to coronary angiographic changes

Patient material

The case material consists of 100 male and 11 female patients, examined at the

University Hospital (clinical care at the Departments of Thoracic Surgery and Internal Medicine), almost all during the years 1963—1966 The male patients were 33—66 and the female patients 44—65 years old For inclusion in the material they should have had a history of typical or suspected angina pectoris, and eventually have been given a clinical diagnosis of coronary artery disease A further condition was that the patients should have successfully undergone *coronary angiography, the work function test* and other examinations described under 'Methods' There were no other dominating diseases, such as severe arterial hypertension, valvular heart disease, pulmonary disease or haematological or metabolic abnormalities of noteworthy degree One male patient had had mild diabetes for 7 years, and 32 male patients had moderate arterial hypertension (with a resting arterial blood pressure within 175—200 mm Hg systolic, or 100—110 diastolic, or 115—135 mean, but with no value above these upper limits), while 3 male patients had an arterial blood pressure slightly above the stated upper limits A clinical diagnosis of one certain or suspected episode of myocardial infarction had been made earlier in 37 male and 6 female patients while two earlier infarctions had been diagnosed in 11 male patients and 1 female, and three infarctions in 7 male patients None of the patients had been in right heart failure, and all had sinus rhythm, but 17 male and 2 female patients had been on digitalis medication before admission to the University Hospital and this treatment was continued unchanged during the examination period The patients were examined and evaluated for possible surgical treatment, and 55 male and 3 female patients were later operated on by the Beck or the combined Beck and Vineberg procedures, as partly reported earlier (24) In the majority of cases, repeated clinical examinations and work function tests were performed during the course of an observation period of several years

Methods and evaluation criteria

Electrocardiography

Leads I, II, III, aVR, aVL, aVF, and V_1—V_6 or V_1, V_2, V_4, V_5 and V_7, were routinely recorded (by the Mingograph 42 or 81, Elema Schonander Ltd, Stockholm) at rest (supine), during an orthostatic test (standing) and after a work test (supine) During the work test (sitting) leads CH_1, CH_2, CH_4, CH_5 and CH_7 were used (with the forehead as reference electrode) The rest Ecg was classified as follows (0) normal or unsignificant change, (1) borderline between normal and abnormal, (2) 'unspecific' abnormal changes such as ST—T depression, signs of left ventricular hypertrophy (3 cases), right bundle branch block (1 case) or left (2 cases), (3) specific signs of earlier infarction i e abnormal Q or QS, or a combination of localized ST elevation and T negativity, or both The localization of ST—T depression during acute coronary insufficiency, or of signs of earlier infarction, was classified as follows (S) 'septal', V_1—V_2 or V_1 and II —III, (A) 'anterior' V_2—V_4 or V_4—V_5, (L) 'lateral', V_5—V_7 or V_6—V_7, (P) 'posterior', II—III and aVF

Orthostatic test and work test

The orthostatic test (8 min standing) and graded work test (bicycle ergometer, stepwise increased load) were performed in principle according to Sjostrand (40 41) and Wahlund (45), as described from this laboratory by *Hallen* (24) *and* Cullhed (12) among others A physician was always present. The precordial Ecg was constantly monitored and intermittently or continuously recorded. The arterial blood pressure was measured, by the usual indirect cuff method, in the *right upper arm at* heart level taking care that the arm was muscularly relaxed The patient's symptoms were recorded, and chest pain during the test was classified, firstly according to *type* [considering character site, and time course] as follows (1) pain reaction not typical of angina pectoris (2) borderline symptoms, suspect or almost typical of angina pectoris (3) typical angina pectoris and secondly according to *degree* (a) slight attack (b) moderate attack (c) severe attack. A work test was planned to be extended to a heart rate of about 170 beats min in younger patients and about 150 in older patients but in the majority of cases the test was terminated earlier because of angina pectoris or Ecg changes typical of coronary insufficiency or both.

The Ecg during and after exercise was classified according to *ST change* during or after work as follows (+) ST elevation of more than 1 mm (1 mm = 0 1 mV) (0) no change of ST junction outside ± 1 mm (1) *junctional* ST depression of 1 5—2 5 mm (measured in 0 5 mm units) *or no junctional* change but change in form (segmental depression, flat or sagging of ischaemic' type), (2) junctional ST depression of *3 mm or more,* or 1 5—2 5 mm depression with form change, (3) junctional ST depression of 3 mm or more and form change The work test Ecg was further classified according to *T change,* as follows (+) more than 1 mm elevation of T during work, (0) no or moderate flattening of T during and after work, the T wave restituting after work in parallel with the successive decrease *in heart* rate once the immediate post test vagal T wave elevation had disappeared, (1) transient depression of T *to isoelectric* ± 0 5 *mm or diphasic after* the end of work, with a typical time course, becoming progressively *more* obvious during the first few minutes after work when the patient has lain down, and then usually disappearing within a further 2—3 minutes or sometimes more, without parallelity with heart rate decrease (2) similar transient depression of T to 1—2 mm negative (3) similar transient depression of T to more than 2 mm negative These criteria applied typically to cases with a normal Ecg at rest the changes of ST and T during coronary insufficiency being usually localized to a few neighbouring precordial leads or leads II—II When the Ecg at rest is abnormal such a classification is more difficult to apply but was used here *in the sense of change from level* of ST or T at rest

Arrhythmia during a work test was classified as (1) supraventricular or infrequent monotopic ventricular extrasystoli (2) heterotopic or frequent monotopic ventricular extrasystoli

Coronary angiography

The patients received no general anaesthesia but were as a rule slightly sedated with 25—50 mg of pethidine and 50 mg of promethazine Nitroglycerine, 0 5 mg, was given sublingually immediately before the injection of the contrast medium A gray straight tip Ödman Ledin catheter, with end and side holes, was introduced percutaneously from the femoral artery into the aortic root by the Seldinger technique The catheter was placed with its tip in the non-coronary sinus The patients were placed prone in the anterior oblique position on a biplane roll film changer (Elema-Schonander Ltd, Stockholm) Urografine (76 %) or Isopaque (60 %), in a dose of 0 7—1 0 ml/kg body weight, was injected at a rate of approximately 35 ml/sec The injection was triggered by Ecg to start at the beginning of a diastole High output roentgen tubes with a focus of 1 2 mm were used The film focus distance was 80 cm, and the exposure factors were 800 mA, 0 01—0 04 sec and 65—85 kV The exposures were made in apnoea after voluntary inspiration, but a Valsalva manoeuvre was avoided as far as possible Linear grids with a 10 1 ratio and high speed high contrast film were used The definition of this full size angiocardiographic system is approximately four periods per mm

The findings on the coronary angiograms were classified, principally according to the status of the main arteries, as follows The right coronary artery (R), the left anterior descending artery (LD) and the left circumflex artery (LC) were considered separately (0) no visible change or only very slight irregularities in the walls, (1) narrowing of the lumen in at least one place but by less than 50 per cent of the maximum diameter of that artery, (2) narrowing of the lumen in at least one place by more than 50 per cent of the maximum diameter, but no delay in filling of the arterial branches distal to the obstruction, (3) as (2) but with delayed filling distal to the obstruction, (4) total occlusion of artery

The total change of the coronary system was expressed as the sum of the degrees of change of the three main arteries, and this sum could therefore vary between 0 and 12 Isolated or dominant obstruction of the short main stem of the left coronary artery was not observed, but would have been classified as LD plus LC

Other methods

Chest roentgenogram, heart volume determination in the sitting position blood and urine analyses, lung function tests, and other routine examinations were performed according to current methods in this Hospital, as described elsewhere (12, 24)

Results

The following presentation will consist mainly of the results obtained from the 100 male patients The results for the female patients will be described briefly at the end of this section

Ecg reaction to work test

The evaluations of the Ecg reactions to work test are exemplified in the Figures Although the present report does not

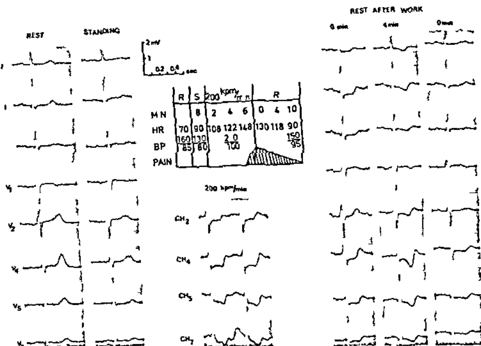


Fig 1 Specific and usual work test reaction in coronary insufficiency with typical time course of ST depression and T inversion in Ecg and with typical angina pectoris. Male patient, 52 years old angina pectoris since age 38 no evidence of infarction heart volume 470/310 ml/ml per m² BSA) no digitalis E aluation of work test reaction ST (depression of ST junction and segment during work) 3 T (depression or inversion of T wave after work 3 ES (extrasystole or other arrhythmia during work) = 0 block (atrio-ventricular or intraventricular conduction disturbance) = 0 AP (angina pectoris) = 3 Evaluation of coronary angiography R (right coronary artery) – 3 LD (descending branch of left coronary artery) – 2 LC (circumflex branch of left coronary artery) = 2

analyse the problem of false positive responses our experience is that an Ecg reaction of the type and degree shown in Fig 1 (classified as summed degree 6 is only met in coronary insufficiency and therefore may be termed not only true positive but also specific It was also a common type of reaction (typical in our cases (Tab I) although variable in degree The degree of specificity is obviously related to the summed degree of the Ecg changes. Ecg reactions with a summed degree of 1 or 0 32 per cent in our series) certainly represent negative responses while a reaction of degree 2 (13 per cent in our series) usually is borderline

The *typical Ecg reaction* in coronary insufficiency is well known from the literature In our cases the following details may be emphasized The time course was almost invariably that the ST depression had its maximum during the final part of the work test and returned within one or a few minutes after the end of work to the resting or control level Exceptions to this rule were only found when after work leads II and

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The total change of the coronary system was expressed as the sum of the degrees of change of the three main arteries, and thus sum could therefore vary between 0 and 12 Isolated or dominant obstruction of the short main stem of the left coronary artery was not observed, but would have been classified as LD plus LC

Other methods

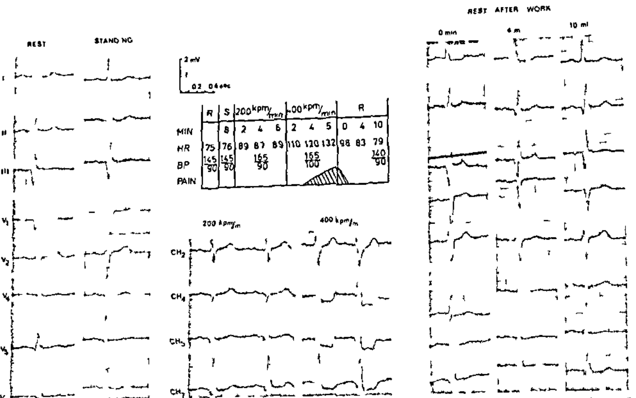
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	R	S	200 kpm/min			400 kpm/min			R		
MIN		8	2	4	6	2	4	5	0	4	10
HR	75	76	89	87	89	110	120	132	98	83	79
BP	145/90	145/90		165/90			165/100				140/90
PAIN											

Fig 2 *Specific* but unusual Ecg reaction in coronary insufficiency Male patient 60 years old angina pectoris since age 44 evidence of (anterior?) infarction at age 47 heart volume 975/500 no digitalis Work test ST = 3 T = 0, IS = 0 block = 0 AP = 3 Coronary angiography R = 2 ID = 4 LC = 2 Abbreviations see Fig 1

TABLE I Male patient material ordered in groups 0—6 *according to Ecg reaction at work test* Depression of ST junction and segment during work (or *after work*, if more pronounced as was exceptionally the case), depression or inversion of T wave transiently after work, extrasystoli during work, T elevation during *work*, digitalis medication, and changes of rest Ecg are presented as number of patients per Ecg reaction *group*

Ecg reaction at work test ST+T change	No of pat	ST depression *during work*				T wave *depression after work*				Extrasystoli during *work*		T wave increase during work	Digitalis medication	*Ecg at rest*			
		0	1	2	3	0	1	2	3	1	2			0	1	2	3
0	14	14				14				2	1	10	4		3		11
1	18		18			18				2		6	3	3	2	6	7
2	13		2	11		11	2			2		4	3	1	3	2	7
3	19		1	10	8	8	10	1		4		6	1	5	4	5	5
4	9			5	4		4	5		2		4		2	1	4	2
5	12			2	10			10	2	1	1	3	5		1	8	3
6	15				15				15	4		4	1	4	3	3	5
Sum	100	14	21	28	37	51	16	16	17	17	2	37	17	15	17	28	40

III showed considerable ST depression, and in such cases the usualy precordial leads V_1—V_7 recorded during work were probably not representative of the affected myocardial area In the great *majority of our cases the ST depression* included form variation ('segmental' or 'ischaemic' depression) Usually, slight depression of the ST junction occurred first followed by the form change, but in some cases these two types of change occurred simultaneously The time course of the T wave change included flattening or a diphasic appearance during the final part of the work test — except when an inverse reaction with T wave elevation appeared as described below — followed by an immediate and transient elevation after the end of work, when the patient had lain down, this was followed by a progressive negativity with a maximum about 2—4 *minutes* after the end of work, with subsequent return to the resting, control level within a further 2—10 minutes The average ratio of degree ST depression at the end of *work to that immediately after work* was 1 84/1 14 in the 100 *male patients* With our evaluation scale, the degree of ST depression during work was almost always greater than the degree of T depression after work, the average ratio being 1 84 0 99 In several cases only the changes during work were noticeable (Fig 2)

The *localization* of the ST depression and the T depression was the same In our series, the localization was S ('septal') in none of the cases, A ('anterior') in 63 per cent, L ('lateral') in 31 per cent, and P ('posterior') in 8 per cent (in a few cases there was overlapping

n lud ng angina pectoris ordered n groups 3—11 according o summed g oss changes w h ao tic sije on No cases fell n g oups 0—2 or 12 degrees of changes Chest pain n ers on to po u y n cases w th a nega e T at rest occurren e of extrasys o belo g ng to t e d fferent angiocard ography groups Cri eria of d ff rent groups are

Ecg at rest 0	1	2	3	T wa e n crease during work	Ex rasys ol during wo k 0	1	2	D gi talis med ca on
			3	2	2		1	1
	2	2	3	4	6	1		2
1	3	2	3	2	9			
2	5	4	4	5	14	1		4
4	2	7	3	6	14	2		1
5	3	6	12	9	18	8		4
2	1	3	7	6	9	5	1	3
	1	2	3	1	6			1
1			2	2	3			1
15	17	28	40	37	81	17	2	17

able ST — T dep ess on during work e en f t e Ecg at rest is not influenced 35 but accord ng to our experience uch depression is related to the ncrease n heart rate and u ually d sappears after ork n parallel with the decrease n heart rate i hout sho ng the different m course of T depress on included in the typ cal E g reaction Except onally ho e er the typ cal Ecg reaction may be se n after d g oxi (e g case no 55 p 9 in Nordstrom—Öhrberg s report) It t erefore seems perm s ble to as ume t at n p te of d g tal s med cat on an Ecg reaction to ork may show a specif c e ourse and gi e support to the d ag o s of co o ary n uff c ency (F g 5 I our Tables the Ecg react ons n pa ents f d g tal med cat on ha e een clas f d under t s assumpt on In one case the rest Ecg sho ed s gns of l ght pre-exc tat on (accelerated con duction) h ch decreased during the orth ostat c test but returned during ork (F g 6) The ST depres on was how ever out of proport on to the degree of pre-exc ta on and as therefore re garded as a s gn of coronary i uff c en cy The Ecg at rest l o ed n one case right bundle-branch block and in 3 cases probable s gns of left ventricular h per troph l le he Ecg react on during exerc se as e aluated as *pos i e for coronary n uff c ency In 2 cases t e* Ecg at rest showed left bundle branch block th a negati e o k react on

A negat e Ecg react on (degree 0—1) as observed n 32 *pat ents i e 32* per ce t (degree 0 n 14 *per cent* and de ree 1 in 18 *per cent In 8 cases no*

Table II Total male patient material of 100 cases of *coronary heart disease with a case history* in the three main coronary branches, as estimated at two-plane coronary angiography Ecg reaction at work test, changes of rest Ecg, appearance of T wave elevation or during work, and presence of digitalis medication are presented as number of patients given in Methods

Cor angio degree	No of pat	Chest pain during work				Ecg reaction in work test						
		0	1	2	3	0	1	2	3	4	5	6
3	3		1		2	1					1	1
4	7	2	1	1	3	2	1	2	2			
5	9	1		5	3	1	3	3		1	1	
6	15	4	1	4	6	3	2	2	5	1	2	
7	16	1	1	1	13		2		4	2	4	4
8	26	3	2	7	14	1	4	3	7	3	2	6
9	15	2		2	11	4	3	3		1	2	2
10	6			1	5		2		1	1		2
11	3			1	2	2	1					
Sum	100	13	6	22	59	14	18	13	19	9	12	15

Arrhythmia or intraventricular conduction disturbance during work (Fig 3) was uncommon in the present series (Tab I, II) The occurrence of ventricular extrasystoli was apparently unrelated to any of the recorded clinical or laboratory factors When extrasystoli appeared, this was always during work and they seldom persisted after the end of work

The *reproducibility* of the Ecg reaction to the work test was not analysed in the present study, but many of our patients were examined more than once and in these cases the reproducibility seemed to be excellent (cf 4)

Atypical Ecg reactions were observed frequently in the 100 male patients In 10 per cent, the rest Ecg showed specific signs of previous infarction In the majority of these latter cases, 23 per cent of the total, an 'inverse' T wave increase appeared during work (Tab I, II), often even at the beginning of the work test, and often so that a localized negative T became positive and sometimes even normal This reaction (Fig 4) was sometimes, but usually not combined with a later appearance of typical ST depression during the final part of work and a transiently more negative T after the end of work A more uncommon reaction in these cases was an elevation of ST during work, appearing in those leads where the infarction residues were localized In 17 per cent the patients were receiving digitalis medication (usually digoxin) often in a low dose at the time of examination It is well known that digitalis medication may cause consider

RES AF ER WORK

0 m 4 m 0 m

R S S AND NG

2 mV

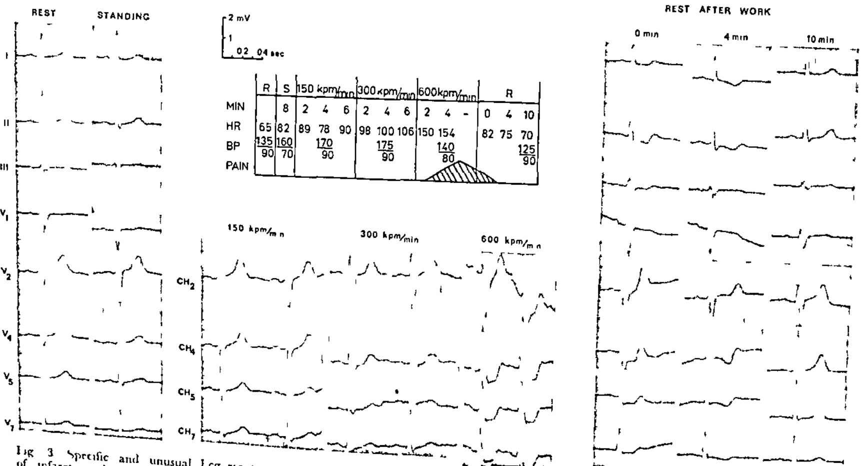
02 04

	R	S	200 kpm/min			400 kpm/min			600 kpm/min			R		
MIN		8	2	4	6	2	4	6	2	4	5	0	4	0
HR	70	03	09	31	3	33	37	3	63	17	177	20	105	04
BP	30/90	40/110	60/110			180/110			195/110					130/10
PAIN														

200 kpm/m 400 pm/m 600 pm/m

V V2 V4 V5 V CH2 CH4 CH5 CH

I g 4 Us al I cg react n of ncertan sj c fc y l ri ng k tes n p a e t w l nf rct res dues i rest I cg Male p a ent 38 years f age a g a j c s af er n er r farc at age 37 I e r lun e 650 420 n I g al s Rest I cg a yp cal R progress o a d nega e I as af er anter r i fa c o Wo k est d g ork there s sl gl t S1 ele a an l lef e I wa e p s y in leads CH5— CH IS = 0 bl ck = ve r c d c on l s rbance Al 3 wl le af er k tler appears lepres o f S1 (= 1) and trans e t I t ga ty (I = 2) le ls II III as n c r ary is ec cy C ro y a g g ap y R = 1 I D = 1 I C = 1 All e n o s see I g I



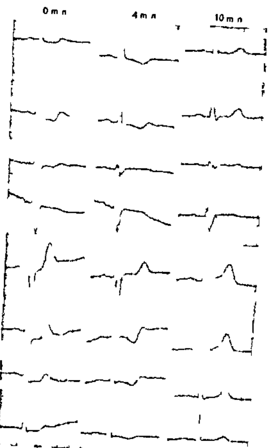
	R	S	150 kpm/min			300 kpm/min			600 kpm/min			R		
MIN		8	2	4	6	2	4	6	2	4	-	0	4	10
HR	65	82	89	78	90	98	100	106	150	154		82	75	70
BP	135/90	160/70		170/90			175/90			140/80				125/90
PAIN														

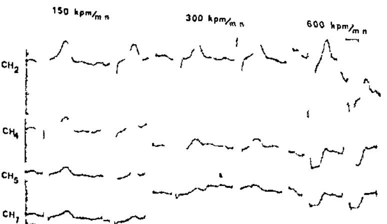
Fig 3 Specific and unusual Ecg reaction in coronary insufficiency Male patient 40 years old angina pectoris since age 30, no evidence of infarction heart volume 930/510 no digitalis Work test SI = 3 I = 3 IS = 0, block = i ventr conduction disturbance, AP = 2 Coronary angiography R = 2 ID = 4 LC = 2 Abbreviations see Fig 1

REST AFTER WORK

	R	S	200 kpm/min			400 kpm/min			600 kpm/min			R		
MIN		8	2	4	6	2	4	6	2	4	5	0	4	10
HR	70	03	09	113	113	33	137	1+3	163	174	177	120	105	104
BP	30/90	0/10	1n0/110			180/110			95/110					130/110
PAIN														

I g 4 Usual Lcg react n of uncerta n specif c ty dur ng work test in pa ent with infarct on res dues in rest Ecg Male pat ent 38 years f age a g a pector s aft r a ter r infarct at age 37 heart vol n e (50/420 no d g tal s Rest Ecg atyp cal R progress n and regative T as af er anter or infarct on Work test dur g work there is sl gl t ST elevat on and def n te T wave pos tivity n leads CH₂—CH₄ IS 0 bl ck = i ventr c nduct on d sturbance M 3 w l le af er work there appears depress on of ST (— 1) and trans ent T negativity (T = 2) n leads II—III as n c ro ary ins ff c ency Coronary a g ography R = 1 ID = 4 IC = 1 Abbrev at ons see F g 1





	R	S	150 kpm/min			300 kpm/min			600 kpm/min		R		
M N		8	2	4	6	2	4	6	2	4	0	4	10
HR	65	82	89	78	90	98	100	106	150	154	82	75	70
BP	135/90	160/70	170/90			175/90			140/80				125/90
PAIN													

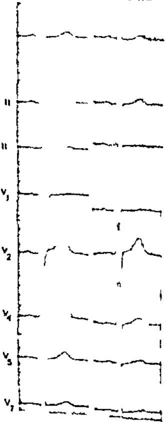


Fig 3 Specif c ... sual ECG react n ... c r ary ... s ff c e cy Male patient 40 years old ang na pector s since age 30 no evidence ... f fa ct ... Heart ... lu ... 930 ... 10 ... igitalis Work test SI 3 I – 3 IS = 0 block = i ventr c nduct n d sturbance AP 2 (r ary ar s ... R 2 (D) 4 LC 2 Abbrev at o s see Fig 1

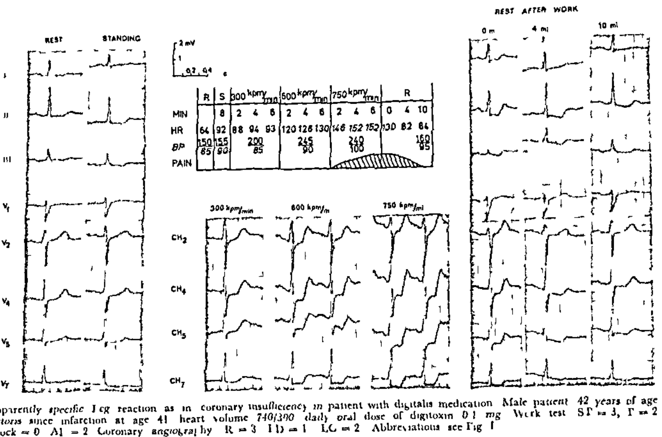
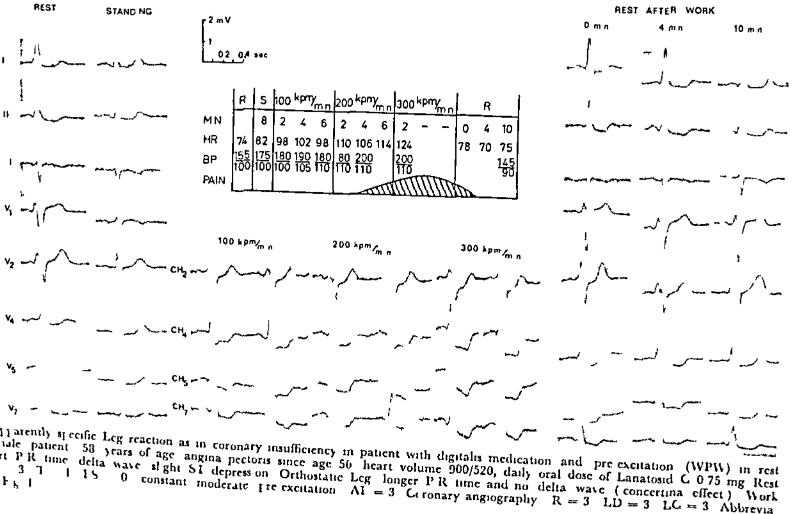
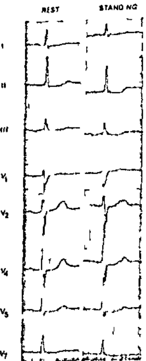


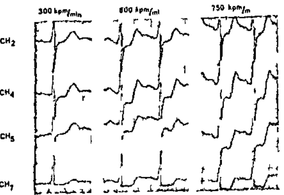
Fig. 6 Apparently *specific* Ecg reaction as in coronary insufficiency *in* patient with digitalis medication Male patient 42 years of age angina *pectoris* since infarction at age 41 heart volume *740/390* daily oral dose of digitoxin *0.1 mg* Work test ST = 3, T = 2 IS = *0* block = 0 AI = 2 Coronary angiography R = 3 ID = 1 LC = 2 Abbreviations see Fig I



	R	S	100 kpm/min			200 kpm/min			300 kpm/min			R		
MIN		8	2	4	6	2	4	6	2	–	–	0	4	10
HR	74	82	98	102	98	110	106	114	124			78	70	75
BP	155/100	175/100	180/100	190/105	180/110	80/110	200/110		200/110					145/90
PAIN														

Fig 5 Apparently specific Ecg reaction as in coronary insufficiency in patient with digitalis medication and pre excitation (WPW) in rest Ecg Female patient 58 years of age angina pectoris since age 56 heart volume 900/520, daily oral dose of Lanatosid C 0 75 mg Rest Ecg short PR time delta wave slight ST depression Orthostatic Ecg longer PR time and no delta wave (concertina effect) Work test ST 3 T 1 IS 0 constant moderate pre excitation AI = 3 Coronary angiography R = 3 LD = 3 LC = 3 Abbreviations see Fig 1





	R	S	300 kpm/min			600 kpm/min			750 kpm/min			R		
MIN		8	2	4	6	2	4	6	2	4	6	0	4	10
HR	64	92	88	94	93	120	126	130	148	152	152	130	82	84
BP	150/85	155/90	200/85			245/90			240/100					160/95
PAIN														

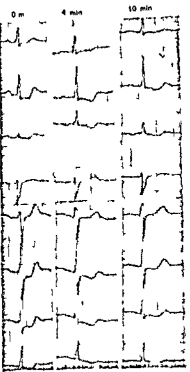
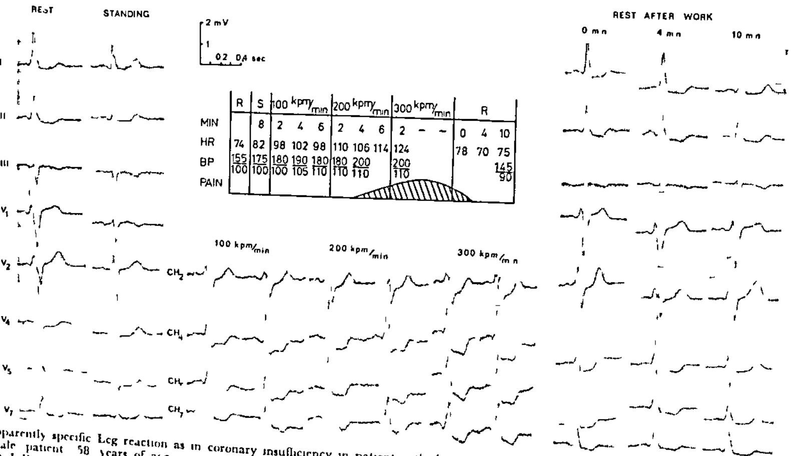


Fig. 6 Apparently specific Leg reaction as in coronary insufficiency in patient with digitalis medication Male patient 42 years of age angina pectoris since infarction at age 41 heart volume 740/390 daily oral dose of digitoxin 0.1 mg Work test SF = 3 T = 2 IS = 0 block = 0 AI = 2 Coronary angiography R = 3 ID = 1 LC = 2 Abbreviations see Fig 1



	R	S	100 kpm/min			200 kpm/min			300 kpm/min			R		
MIN		8	2	4	6	2	4	6	2	–	–	0	4	10
HR	74	82	98	102	98	110	106	114	124			78	70	75
BP	155/100	175/100	180/100	190/105	180/110	180/110	200/110		200/110					145/90
PAIN														

Fig. 5 Apparently specific Ecg reaction as in coronary insufficiency in patient with digitalis medication and pre excitation (WPW) in rest Ecg. Female patient 58 years of age angina pectoris since age 56 heart volume 900/520 daily oral dose of Lanatosid C 0.75 mg. Rest Ecg short PR time delta wave slight ST depression. Orthostatic Ecg longer PR time and no delta wave (concertina effect). Work test ST 3 T 1 TS = 0 constant moderate pre excitation AI = 3. Coronary angiography R = 3, LD = 3, LC = 3. Abbreviations see Fig 1

for a few minutes after work a type of blood pressure reaction which was noted in several other patients in this series.

Chest pain at work test

In the work tests typical angina pectoris occurred in 59 per cent, and suspected or almost typical in 22 per cent, while chest pain not typical of angina pectoris occurred in 6 per cent and no pain in 13 per cent (Tab II) The atypical chest pain always was the usual type of distress experienced by the individual patient, e g oppression with slight pain although the time course or the localization might be atypical Their case histories had usually been interpreted as suspected but not typical angina pectoris

The work test offered a possibility of substantiating the case history and analysing the angina pectoris The time course of the angina usually showed an increase from the first faint feeling to moderate intensity in 1—3 minutes exceptionally as quickly as in half a minute or as slowly as in about 5—10 minutes After the end of the work test the pain usually disappeared during the first few minutes and seldom persisted for more than 5 minutes The character of the angina usually changed somewhat during the increase of symptoms e g from a feeling of numbness or coldness to the typical oppressive or burning pain The localization was usually first the sternal area spreading later to the left shoulder and upper arm also eventually to the left hand right arm or neck, often with different types of sensation in different regions In a few exceptional cases the pain started in the left upper arm the apical left chest region or the right part of the chest

In 13 cases *no chest pain* at all occurred at the work test As described above, in 8 of these cases the Ecg reaction was also negative In the remaining 5 cases (patients no 42, 47, 81, 82, 89), the Ecg reactions to work were classified as degrees 3, 2 6, 5, 2 in 2 of these cases the rest Ecg showed specific signs of infarction in 2 cases unspecific ST — T depression and in 1 case right bundle branch block, the coronary angiograms were classified as 7, 4 8, 8, 6 degrees, and the heart volumes were 830/400 1960/1140, (aneurysmal dilatation of left ventricle after infarction ½ year before examination) 990/570 750/390, 980/520 and the work tests were discontinued at W_{max} 900, 370 170 800 800 kpm min at heart rates of 148 130, 116 161 164

Coronary angiography

Coronary angiography usually revealed changes in all three large coronary branches (Tab II III), of variable degree and distribution (Tab III) Only 11 of the 300 visualized branches were judged to be without luminal changes and 54 branches in 48 patients were judged *to be totally occluded* As has been reported earlier one major coronary branch may be totally occluded even though there is no clinical or no specific electrocardiographic evidence of earlier infarction This was observed in 17 of the 42 cases with one branch totally occluded and in 8 of these cases the rest Ecg was normal in 3 cases borderline and in 6 cases unspecifically abnormal while the Ecg reaction to

chest pain or angina pectoris occurred at the work test In 3 of these (no 16, 34, 100), age 43, 49, 63 years, the Ecg at rest was borderline and the angiocardiogram showed moderate coronary changes (degrees 6, 5, 6), while the work test was discontinued after maximum loads (W_{max}) of 600, 800, 570 kpm min at heart rates of 126, 150, 118 beats min because of general tiredness and breathlessness, so it is quite possible, and in our interpretation will be assumed, that coronary insufficiency was not provoked at the work test Of the remaining 5 cases without angina pectoris, of ages 46—53 years, all had an earlier clinical diagnosis of infarction, the Ecg at rest showed considerable changes (3 cases specific infarction residues, 1 case negative T wave, 1 case left bundle branch block), the angiocardiogram showed degrees 4—9, the work test was interrupted at W_{max} 470—630 and at heart rates of 140—176 because of moderate tiredness and breathlessness in the patients and for reasons of caution In these 5 cases also, coronary insufficiency may not have developed, but because of the altered Ecg at rest, the negative Ecg reaction at work was not taken as evidence against coronary insufficiency The T wave in 3 cases showed inverse elevation during work

In 22 cases of negative Ecg reaction, typical angina pectoris occurred at the work test and for this reason the test was discontinued In 2 additional cases, chest pain appeared, being the usual type of pain in these patients which was not typical of angina pectoris but was still considered as consistent with coronary insufficiency In 19 of these patients, including those with atypical pain, the Ecg at rest showed considerable changes (in 17 cases specific infarction residues, in 1 case left bundle-branch block, in 1 case left ventricular hypertrophy), and during work an inverse T-wave elevation occurred in 15 cases In these cases also, the negative Ecg reaction to work was not interpreted as evidence against coronary insufficiency There remained 5 patients (no 30, 33, 43, 68, 72) who had typical angina pectoris at work, a normal or borderline rest Ecg, and a negative Ecg reaction to work (ST depression of degree 0 in case no 72, degree 1 in the other cases) They all had an arterial blood pressure within normal limits, and no evidence of earlier infarction

Patient no 72 was 57 years old heart volume 950/550 (ml/ml per m²) coronary angiogram R = 4, LD = 2 LC = 3, highest work load 200 kpm/min at heart rate 114, typical angina pectoris of moderate intensity, heterotopic ventricular extrasystoli He was operated on by the Beck Vineberg procedures 2 months later, and re examined 15 months after the operation with essentially the same work test reaction (ST depression degree 1, angina pectoris and heterotopic VES at 200 kpm/min) Patients no 30 33, 43 and 68 were 47—51 years old their heart volumes were 850/410, 950/410 740 380 700/380 the angiograms showed degrees (R LD LC) of 1 3 3 1 1 3 4 2 2 4 4 3 the work test was discontinued at W_{max} 670 600, 500 300 kpm/min and at heart rates of 148 136, 140 120 Patient no 68 thus had very advanced coronary changes a slight junctional ST-depression (degree 1 was only apparent in leads II and III after work Patient no 43 showed a very steep rise of the arterial blood pressure in parallel with the onset of angina pectoris during work with a maximum systolic value of 260 mm Hg and an increased diastolic level

TABLE IV Male patient material ordered in groups according to coronary angiography (as in Table II) year of birth (as in Table VII) and highest work load (as in Table V) Heart volume in the sitting body position is presented as average total volume and as average volume per m² body surface area

Cor angio degree	No of pat	Heart volume ml/ml per m²	Year of birth	No of pat	Heart volume ml/ml per m²	W_{max} kp/min min	No of pat	Heart volume ml/ml per m²
3	3	1043/537	1896—1900	3	900/447	1—100	2	1230/600
4	7	1129/601	1901—1905	13	881/462	101—200	8	849/478
5	9	892/442	1906—1910	28	929/495	201—300	21	918/509
6	15	919/479	1911—1915	21	940/507	301—400	13	895/488
7	16	863/450	1916—1920	19	978/486	401—500	18	961/493
8	26	885/463	1921—1925	10	836/437	501—600	20	899/460
9	1	917/502	1926—1930	5	880/476	601—700	8	910/469
10	6	932/470	1931—1935	1	690/390	701—800	8	849/440
11	3	983/550				801—	2	900/425

Work capacity

The Sjostrand—Wahlund work function test measures that type of capacity for physical work which is primarily limited by circulatory functional capacity In normal individuals the result may be expressed simply as oxygen pulse or work pulse e g as work load (W) at a given heart rate (e g 170 150 or 130) in steady state In a normokinetic state measures such as W_{170} or W_{130} are therefore principally related to cardiac stroke volume and — in the normal individual to maximal oxygen uptake and maximal work capacity In a group of patients such as the present series there is abnormal limitation of work capacity by coronary insufficiency and the results of the work test therefore have to be expressed as observed W_{130} or a similar measure as observed W_{max} being the highest load actually performed up to the limit set by the coronary insufficiency slightly recalculated so as to correspond to the load which the patient would actually be able to perform for about 6 minutes, in the way suggested by Strandell (43) and as HR_{max} being the highest heart rate actually reached When evaluating HR_{max} in a patient, the average predicted maximal heart rate in a normal individual of the same age should be considered In addition the total work performed in the work test (W_{tot}) was calculated

The work capacity expressed as observed W_{max} varied considerably in the present material (Tab V VI) There was a tendency for patients with a very high summed degree (9—11) of angiographic coronary changes to have a lower W_{max} than the rest of the patients With more moderate coronary changes (degrees 3—8) no such tendency was observed the variation was large, and individual prediction was therefore not possible with any reasonable precision

The reproducibility of W_{130} and W_{max}

TABLE III Male patient material ordered in groups according to summed gross coronary changes at angiocardiography, as in Table I Localization of gross angiographic changes in the three large coronary branches are presented as number of patients belonging to the summed groups

Cor angio degree	No of pat	A coron d cor ang degree 0	1	2	3	4	A coron sin r desc cor ang degree 0	1	2	3	4	A coron sin r circ cor ang degree 0	1	2	3	4
3	3	1	2				2	1					1	1	1	
	7	3	4					1	4	1	1	1	5		1	
5	9	1	5	2	1			4	2	2	1	1	3	3	2	
6	15		5	4	4	2		6	3	2	4	1	8	3	3	
7	16		2	8	6		1	1	8	4	2		1	8	6	1
8	26			9	8	9			15	7	4		2	12	11	1
9	15			3	4	8			7	3	5		2	2	8	3
10	6					6				5	1			1	5	
11	3					3				1	2				2	1
Sum	100	5	18	26	23	28	3	13	39	25	20	3	22	30	39	6

exercise was negative in 1 case, both the Ecg and pain reactions negative in 1 case, and the pain reaction negative in 1 case Even in one of the 6 cases with two major branches totally occluded (case no 68), where the third branch was classified as almost occluded (degree 3) evidence of infarction was absent, the rest Ecg normal and the Ecg reaction to work was negative although typical angina pectoris occurred The latter patient was operated on by the Beck procedure he was reexamined 5 years after operation and then showed a slight increase of W_{max} (from 300 to 370 at a heart rate of 118), and unchanged Ecg and pain reactions On the other hand the three patients with a summed angiographic degree as low as 3 all showed specific signs of infarction in their rest Ecg 2 of them had positive Ecg and pain reactions to work while 1 patient had a negative Ecg reaction and unspecific chest pain

Heart volume

There was a tendency to increased heart volume in the patients as a group The heart volume measured in the sitting body position was thus within the range 500—600 ml/m body surface area (corresponding total volumes of 890—1150 ml) in 24 cases within 600—800 ml m² (1050—1730 ml) in 6 cases and within 800—1200 ml/m² (1550—1960 ml) in 3 cases two of whom had aneurysmal dilatation of the left ventricle If the last mentioned 3 cases are disregarded there was no consistent relationship in the present material between on the one hand heart volume and on the other summed degree of coronary angiography age or highest performed work load (Tab IV)

TABLE IV Male patient material ordered in groups according to coronary angiography (as in Table II) year of birth (as in Table VII) and highest work load (as in Table V) Heart volume in the sitting body position is presented as average total volume and as average volume per m² body surface area

Cor angio degree	No of pat	Heart volume ml/ml per m²	Year of birth	No of pat	Heart volume ml/ml per m²	W_{max} kp min min	No. of pat.	Heart volume ml ml per m²
3	3	1043/537	1896—1900	3	900/447	1—100	2	1230/600
4	7	1129/601	1901—1905	13	881/462	101—200	8	849/418
5	9	892/442	1906—1910	28	929/495	201—300	21	948/509
6	15	919/479	1911—1915	21	940/507	301—400	13	895/488
7	16	863/450	1916—1920	19	9[illegible]8/486	401—500	18	961/493
8	26	885/483	1921—1925	10	836/437	501—600	20	899/460
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Work capacity

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The work capacity expressed as observed W_{max} varied considerably in the present material (Tab V VI) There was a tendency for patients with a very high summed degree (9—11) of angiographic coronary changes to have a lower W_{max} than the rest of the patients With more moderate coronary changes (degrees 3—8) no such tendency was observed the variation was large and individual prediction was therefore not possible with any reasonable precision

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Table III Male patient material ordered in groups according to summed gross coronary changes at angiocardiography, as in Table I Localization of gross angiographic changes in the three large coronary branches are presented as number of patients belonging to the summed groups

Cor angio degree	No of pat	A coron d cor ang degree					A coron sin r desc cor ang degree					A coron sin r circ cor ang degree				
		0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
3	3	1	2				2	1					1	1	1	
	7	3	4					1	4	1	1	1	5		1	
5	9	1	5	2	1			4	2	2	1	1	3	3	2	
6	15		5	4	4	2		6	3	2	4	1	8	3	3	
7	16		2	8	6		1	1	8	4	2		1	8	6	1
8	26			9	8	9			15	7	4		2	12	11	1
9	15			3	4	8			7	3	5		2	2	8	3
10	6					6				5	1			1	5	
11	3					3				1	2				2	1
Sum	100	5	18	26	23	28	3	13	39	25	20	3	22	30	39	6

exercise was negative in 1 case, both the Ecg and pain reactions negative in 1 case, and the pain reaction negative in 1 case Even in one of the 6 cases with two major branches totally occluded (case no 68), where the third branch was classified as almost occluded (degree 3), evidence of infarction was absent, the rest Ecg normal, and the Ecg reaction to work was negative although typical angina pectoris occurred The latter patient was operated on by the Beck procedure, he was reexamined 5 years after operation, and then showed a slight increase of W_{max} (from 300 to 370, at a heart rate of 118), and unchanged Ecg and pain reactions On the other hand the three patients with a summed angiographic degree as low as 3 all showed specific signs of infarction in their rest Ecg, 2 of them had positive Ecg and pain reactions to work while 1 patient had a negative Ecg reaction and unspecific chest pain

Heart volume

There was a tendency to increased heart volume in the patients as a group The heart volume, measured in the sitting body position, was thus within the range 500—600 ml/m² body surface area (corresponding total volumes of 890—1150 ml) in 24 cases, within 600—800 ml/m (1050—1730 ml) in 6 cases, and within 800—1200 ml/m² (1550—1960 ml) in 3 cases two of whom had aneurysmal dilatation of the left ventricle If the last mentioned 3 cases are disregarded there was no consistent relationship in the present material between on the one hand, heart volume and on the other summed degree of coronary angiography or highest performed work load (Tab IV)

W sho ed a cons derable reduct on (Tab V) There is a small systemat c error in the a erage values of W 50 and a larger error for W 0 as in the patients th the lo est HR_{max} the values for W and W1 0 ere not calculated in Tab V This error does not nval date he general trend ho e er

Rea tion to orth otat c te t

At re t n the sup ne po t on before the orthostat c te t and work test the average h art rate as 3 beats min (range 50—101 After 8 m nutes of stand ng the chan e n heart rate as + 13 9 (ranging from — 12 to + 40) In 7 cases there as al o modera e ST junctional depres on T a e flatten ng and appearance of a pos ti e after potent al all be ng a sympath coton c type of react on the r heart rate nc ease as + 24 6 and dur ng exerc e they all had angina pectoris degree 2 9 and a pos ti e Ecg react on deg ee 4 7)

Compl cat ons

There ere no no e orthy complica ons n the present series of exam nat ons No ausculta ory or other cl nical s gns of left en ricular fa lure (pulm onary oe dema ere noted e her during or after o k In a few cases the arterial blood pres ure fell sl ghtly at the e d of the o k est hen coronary nsuff c ency appeared but more of en the reverse r act on occurred w h a abnormally eep ncrea e n pres ure

Ma c al of fema e pat e ts

In add on to the material of 100 male pa ents ho ha e been descr bed abo e a group of 11 female pat ents were full exam ed during the time period in quest on Their average age was 54 years (range 44—65) the coronary angiograms sho ed degree 6 7 (3—10) the rest Ecg had degree 0 in 3 cases, 1 in 1 case 2 n 5 ca es and 3 in 2 cases the Ecg react on to the work test had degree 0 n 2 cases 1 in 1 case 2 n 4 cases 4 n 2 ca es and 5 n 2 cases W_{max} as 250 kpm min (130—450) HR_{max} 128 beats n n (95—158) and W 50 244 kpm m n (130—420) the heart volume was 6 9 408 ml ml per m (480—900 320—570) In one of the 3 cases th a negat ve Ecg react on to the work test there as typ cal ang na pectoris and the rest Ecg sho ed specif c nfarct on res dues n the other t o cases the rest Ecg as norm al and the subjective react on to ork as breath lessness and oppress on in one case a d anx ety n the second case but no pa n In another pat ent there was no pa n *during work but a pos t e Ecg reaction* degree 2 In the rema n ng 7 female pat ents the Ecg react on to ork as pos ti e and angina pectoris appeared.

Discussion

In est ga ons into the val d ty of the Ecg react on to a work test as a s gn of relat ve coronary insuff c ency — def ned as trans en ly and reg onally nadequate oxygen (blood) supply in relation to myocard al oxygen need neet several types of d ff cult es A true answer as to the ex tence of coronary nsuffic ency can ne er be prec sel obta ned partly because *such factors* as relat e coronary nsuff c ency an na pectoris s gns of left ventr cular fa lure ang ograph c

TABLE V Male patient material ordered in nine groups according to highest work load (W_{max}) performed for 6 min at work test Age at *examination* coronary angiographic changes Ecg reaction at work test, highest observed heart rate (HR_{max}) at work test, total work (W_{tot}) performed at test, and duration of test in minutes (T_{tot}) are presented as averages *and ranges for the different* W_{max} groups and refer to all 100 patients In addition, the work loads at heart rate 130 (W_{130}) and 150 (W_{150}) could be calculated by intrapolation or slight extrapolation in part of the *material* (figures within brackets show number of patients included in each average) and are also presented as averages and ranges

W_{max} kpm/min	No of pat	Age years	Cor angio degree	Ecg at work test	HR_{max} beats/min	W_{tot} kpkm	T_{tot} *min*	W_{130} (n) kpm/min	W_{150} (n) kpm/min
1—100	2	53	9	2	109	0 3	2 5	150 (1)	—
		50—55	8—10	1—3	88—130	0 2—0 4	1—4	—	—
101—200	8	56	8 1	3 8	116	1 3	7 8	218 (4)	240 (2)
		48—63	6—11	0—6	88—148	0 7—1 8	6—10	150—330	210—270
201—300	21	54	7 5	2 9	121	2 4	10 8	289 (13)	330 (3)
		36—62	3—11	0—6	86—185	1 8—3 6	8—18	140—570	200—390
301—400	13	48	7 2	3 0	125	3 5	12 0	310 (10)	440 (2)
		33—60	4—9	1—6	93—158	2 4—6 0	7—24	240—600	370—510
401—500	18	53	7 4	2 8	130	5 0	15 3	441 (14)	506 (8)
		39—64	4—9	0—6	88—160	3 6—6 6	9—20	180—700	420—690
501—600	20	50	6 9	2 6	142	6 6	17 3	482 (19)	593 (13)
		38—66	3—10	0—6	115—178	5 1—9 6	12—22	180—780	280—810
601—700	8	43	6 3	2 4	147	7 4	18 5	556 (8)	710 (4)
		37—47	4—8	0—6	121—164	5 2—8 8	13—28	300—900	520—900
701—800	8	52	6 4	3 1	155	10 5	20 1	573 (8)	775 (8)
		38—59	4—8	1—5	136—172	8 1—12 0	15—24	420—710	610—1000
801—	2	56	7	3	158	13 2	21	700 (2)	910 (2)
		51—61	7—7	3—3	148—168	10 8—15 6	18—24	670—730	900—920

was found to be good in those patients who were examined repeatedly (cf 4) In a few cases however, there were large variations without any obvious explanation Standardization of the work test with regard to environmental factors such as time of day relation to meals *etc* was attempted but could not be strictly controlled and such factors as variable mental tension and changing nitroglycerine treatment also introduced uncontrolled errors probably to some degree influencing the result of the work test in such exceptional cases

The HR_{max} amounted on an average to 74—82 per cent of the predicted maximal heart rate regard being taken of age (Tab VII) This is a relatively high percentage figure, with respect to the advanced state of disease in the present patients and was not much lower in older than younger patients With decreasing W_{max} HR_{max} also decreased moderately but at the same time W_{130} or

$W_{1,0}$ showed a considerable reduction (Tab V) There is a small systematic error in the average values of W_{130} and a larger error for $W_{1,0}$, as in the patients with the lowest HR_{max} the values for W_{130} and $W_{1,0}$ were not calculated in Tab V This error does not invalidate the general trend however

Reaction to orthostatic test

At rest in the supine position before the orthostatic test and work test, the average heart rate was 73 beats min (range 50—101) After 8 minutes of standing the change in heart rate was + 13 9 (ranging from — 12 to + 40) In 7 cases there was also moderate ST junctional depression T wave flattening and appearance of a positive after potential, all being a sympathicotonic type of reaction their heart rate increase was + 24 6 and during exercise they all had angina pectoris (degree 2 9) and a positive Ecg reaction (degree 4 7)

Complications

There were no noteworthy complications in the present series of examinations No auscultatory or other clinical signs of left ventricular failure (pulmonary oedema) were noted either during or after work In a few cases the arterial blood pressure fell slightly at the end of the work test when coronary insufficiency appeared but more often the reverse reaction occurred with an abnormally steep increase in pressure

Material of female patients

In addition to the material of 100 male patients who have been described above a group of 11 female patients were fully examined during the time period in question Their average age was 54 years (range 44—63), the coronary angiograms showed degree 6 7 (3—10) the rest Ecg had degree 0 in 3 cases 1 in 1 case, 2 in 3 cases, and 3 in 2 cases, the Ecg reaction to the work test had degree 0 in 2 cases 1 in 1 case, 2 in 4 cases, 4 in 2 cases and 5 in 2 cases, W_{max} was 250 kpm min (130—450), HR_{max} 128 beats min (95—158) and $W_{1,0}$ 244 kpm min (130—420), the heart volume was 679 408 ml ml per m² (480—900/320—520) In one of the 3 cases with a negative Ecg reaction to the work test there was typical angina pectoris and the rest Ecg showed specific infarction residues, in the other two cases the rest Ecg was normal and the subjective reaction to work was breathlessness and oppression in one case and anxiety in the second case but no pain In another patient there was no pain during work but a positive Ecg reaction degree 2) In the remaining 7 female patients the Ecg reaction to work was positive and angina pectoris appeared

Discussion

Investigations into the validity of the Ecg reaction to a work test as a sign of relative coronary insufficiency — defined as transiently and regionally inadequate oxygen (blood) supply in relation to myocardial oxygen need — meet several types of difficulties A true answer as to the existence of coronary insufficiency can never be precisely obtained partly because such factors as relative coronary insufficiency angina pectoris signs of left ventricular failure, angiographic

TABLE VI Male patient material ordered in groups according to coronary changes at angiocardiog or calculated by intrapolation to correspond to this value and total work actually group

Cor angio degree	No of pat	Highest work load in kpm/min performed for 6 min, W_{max}: 0— 100	101— 200	201— 300	301— 400	401— 500	501— 600	601— 700	701— 800	801— or more
3	3			1			2			
4	7			2	2	1		1	1	
5	9			2	1	1	2	2	1	
6	15		1	2	1	1	6	1	3	
7	16		2	3		6	1	2		2
8	26	1	2	3	6	5	4	2	3	
9	15		2	2	3	4	4			
10	6	1		4			1			
11	3		1	2						
Sum	100	2	8	21	13	18	20	8	8	2

TABLE VII Male patient material ordered in 8 groups according to year of birth (the examinations were performed during the years 1963—1966) Coronary angiographic changes highest work load (W_{max}) and highest heart rate (HR_{max}) at work test and predicted maximal heart rate at complete exhaustion in normal subjects are presented as average values

Year of birth	No of pat	Cor angio degree	W_{max} kpm/min	HR_{max} beats/min	maximal HR predict
1896—1900	3	7.0	450	122	155
1901—1905	13	6.0	455	124	160
1906—1910	28	8.3	385	122	165
1911—1915	21	7.1	468	138	170
1916—1920	19	6.8	501	137	175
1921—1925	10	6.7	565	146	160
1926—1930	5	6.0	530	151	185
1931—1935	1	6.0	400	124	190

coronary changes (even total occlusion) episodes of myocardial infarction and observations on structural changes at operation or at some later date at post-mortem are not congruent and may vary independently and partly because one of the two main variables in coronary insufficiency (regional myocardial oxy gen need) is very seldom possible to measure As the true answer cannot be

raphy as in Table II Highest work load which was actually performed for 6 min performed at work test are presented as number of patients per angiocardiographic

Total work in kpkm performed in work test W_{tot}											
0—1	1—2	2—3	3—4	4—5	5—6	6—7	7—8	8—9	9—10	10—11	11 or more
		1			1		1				
	2	1			2			2			
		1	2		3	2					1
	1	2	1	1	1	3	3	2	1		
1	2	2	2	1	4		1	1		1	1
1	3	5	4	1	3	3	1	2			3
	3	2		4	3		2		1		
1		4			1						
	2	1									
3	13	19	9	7	18	8	8	7	2	1	5

precisely stated in the individual case an exact validation of the Ecg reaction to a work test, or any other measure of relative coronary insufficiency cannot be made With this reservation the present results have been interpreted by us as giving probable evidence for the occurrence in about 15 per cent among our cases with a normal or borderline rest Ecg of a false negative Ecg response and in a similar percentage of a false negative pain reaction (cf 4) The true figure of false negative responses in these cases may have been somewhat higher however With the two-step test higher proportions of false negative responses have usually been reported (1 3 11 14 18 25 28—30 34 36—38 46) but the results of these reports cannot be directly compared with the present results for several reasons the present type of work test is different and has considerable diagnostic advantages (recording of Ecg during work graded and successively increased load until a clinical limit is reached) over the two step tests as used in the above reports the principles of classification of the Ecg reaction are different (cf 1 5—7 9 10 13 17) and the clinical materials are different (e g with respect to proportion of patients with a normal rest Ecg) We also draw the conclusion from our results that a condition with a considerably abnormal Ecg at rest especially with infarction residues reduces the validity of the Ecg reaction to the work test so that there are more false negative reactions although the true proportion cannot be demonstrated with any great precision In such advanced cases the pain reaction also gives more frequent false negative response From a practical point of view it is therefore

necessary always to use *both* the Ecg reaction and the pain reaction for the diagnosis of relative coronary insufficiency during a work test, and to consider symptoms and clinical signs during work as especially important in cases with a considerably abnormal rest Ecg In consistence with this conclusion a physician ought always to be present at a work test of that type

There may be several causes of 'false negative Ecg reactions to a work test in patients with a normal Ecg *at rest* Assuming that relative coronary insufficiency really occurred — which may be difficult to prove with reasonable certainty *in the absence of typical angina pectoris* — the reason may have been that the cardiac action currents really did not change possibly because of a diffuse insufficiency throughout the whole myocardium, or that the customary precordial leads were not representative enough The latter possibility seems probable, for the following reason Depression of ST as part of a positive Ecg reaction was localized in posterior leads in only 6 per cent of the cases, while infarction residues in our cases were localized in 'posterior' leads in 35 per cent One should expect posterior' coronary insufficiency to occur more frequently than suggested by the Ecg reactions Additional dorsal thoracic or lumbar leads may therefore be of value in increasing the validity of the Ecg reaction to a work test

The evidence of 'false positive Ecg reactions to a work test is not analyzed in the present investigation Conclusions in the literature on this problem vary considerably (cf 25 33, 36, 46), and it is generally realised that the degree of strictness of the Ecg evaluation criteria *determine* this incidence In general, emphasis has been put on change of ST *form* (to flat or sagging, 'segmental' *depression)* rather than degree of ST depression or T-wave change The optimal 'diagnostic weight' of these three variables obviously has not yet been settled, but our experience, in contrast *to that* of several other investigators, is that a transient T wave negativity appearing *and disappearing usually during* the first to fourth minutes after work may be as specific a sign of considerable coronary insufficiency as a prominent ST segmental depression during work From our results we conclude that repeated Ecg recordings should be made during the last part of the work test and the first few minutes afterwards This has been customary with the Sjostrand—Wahlund work test (cf 7 20 27, 41)

The well known partial discrepancy between structural changes of the three main coronary arteries and clinical or post-mortem evidence of myocardial infarction (2, 15, 16, 19, 22 23, 42) was substantiated in the present series In the present group of patients there was no obvious correlation between localization of infarction residues in the Ecg at rest and site of angiographic change Thus in 28 cases this Ecg localization was anterior and the average angiographic change for the right coronary artery (R) was then 2 46 for the left descending artery (LD) 2 82 and for the left circumflex artery (LC) 2 04 In 11 cases the Ecg localisation was lateral and the angiographic changes R = 2 82, LD = 2 64 LC = 2 36 while

in 23 cases the Ecg *localization* was posterior and R = 2 83 LD = 2 26, LC = 2 35

The localization of the ST depression during the work test did not show any consistent correlation to site of angiographic changes, either In the patients without infarction residues in their Ecg at rest the ST depression showed an terior localization in 46 cases and the angiography showed R = 2 54 LD = 2 28 LC = 2 35 the ST depression was lateral *in 32 cases, and the angiography* showed R = 2 56 LD = 2 31, LC = 2 03 while a posterior ST depression was observed in 6 cases where angiography showed R = 2 83 LD = 3 17 LC = 2 50 *When those patients* were added who had infarction residues in their Ecg at rest the average values for angiography results at different localizations of ST depression were very little changed Thus the site of coronary constrictions at angiography cannot be predicted from the localization of electrocardiographic infarction residues or of ST depression at work

Assuming that the degree of limitation of physical work capacity under the experimental circumstances is a valid measure of critical limitations of coronary perfusion capacity in relation to myocardial oxygen need our results also demonstrate that there is a considerable discrepancy between coronary angiographic findings and critical limitation of coronary perfusion capacity Similar observations in other patient materials have already been reported (31 cf 44) In the present material cases with obviously increased myocardial oxygen demand (e g considerable arterial hypertension or demonstrated aortic stenosis or insufficiency) were not included but still the myocardial oxygen need per performed work must have shown some individual variation in our material This variation *factor could, however* hardly be the major explanation of the discrepancy Probably an individual *variability of the arteriosclerotic* changes in small arterial vessels and of the formation of larger anastomotic vessels, irrespective of the changes in the main arteries is a more important explanation

It is well known that acute coronary insufficiency causes signs of acute left ventricular failure (cf 32, 34) Cardiac enlargement on the other hand, is a late *and inconsistent finding in* C A D *and* is not a reliable measure of advanced disease The present results demonstrate that a decrease in work pulse parallels the decrease in exercise tolerance caused by limitation due to coronary insufficiency As a hyperkinetic circulation (26) is not to be expected in these cases — if anything the reverse — and as the mechanical efficiency in this type of work probably is not *changed* the decrease in work pulse may be interpreted as a sign of a decrease in cardiac stroke volume during exercise The conclusion then should be that when the disease becomes more advanced as judged from the decrease in work tolerance typical indirect signs of myocardial (left ventricular) dysfunction appear in the form of a decreased stroke volume per heart volume This conclusion is tentative and will need corroboration by direct measurements especially of left ventricular stroke volume diastolic volume and diastolic filling pressure It would however be

of considerable practical clinical value, e g in the choice of cases for surgical therapy

The orthostatic reaction was not pronounced in our material (cf 8, 21) This result corroborates our general experience, namely that in C A D cases orthostatic Ecg changes of a 'sympathicotonic' type (26) are less rather than more usual than in normal persons and that the orthostatic test is a very valuable tool in the differential diagnosis of the sympathicotonic orthostatic reaction' and vasoregulatory asthenia' (26)

Summary

A group of 100 male and 11 female patients with relatively advanced coronary heart disease, and a case history including periodic angina pectoris in all cases and evidence of earlier myocardial infarction in 55 male and 7 female patients, were examined clinically and by coronary angiography, by an orthostatic test and by the Sjostrand—Wahlund graded work test including electrocardiography The results were classified and different interrelationships studied

At the work test there were 5 male patients with a false negative' Ecg reaction, in the presence of angina pectoris, although the Ecg at rest was normal or borderline, and 5 male patients and 1 female with a false negative pain reaction in the presence of a positive Ecg reaction There were also 3 male and 2 female patients without either chest pain or Ecg changes at the work test, although the rest Ecg was normal or borderline, and in these cases the test may have failed to provoke coronary insufficiency even though high heart rates were attained In addition, 19 male and 1 female patients had a negative' Ecg reaction at the work test, in the presence of angina pectoris, but in these cases the Ecg at rest was considerably abnormal with infarction residues, left bundle branch block or similar changes, and the Ecg reaction to work difficult to interpret

Depression of ST was in the great majority of cases more pronounced during work than immediately afterwards A flat or negative T wave occurred transiently after work, with a maximum at about 3—4 minutes after the end of the test, but usually only in cases with a considerable ST 'segmental' depression, and therefore may be a sign of more pronounced coronary insufficiency The ST and T changes were generally localized to the anterior or lateral leads of the left precordium, in contrast to the Ecg localization of infarctions in this and other series of patients This observation is consistent with the hypothesis that coronary insufficiency within the posterior wall and septum is more difficult to diagnose during a work test with the present Ecg lead system than within the anterior or lateral wall

Site of coronary arterial changes at angiography was not correlated to localization of Ecg changes either infarction residues or ST depression during work

The physical work capacity (work tolerance) was in the first hand expressed as W_{max}, the highest load which could actually be performed on the bicycle ergometer for about 6 min before limitation by coronary insufficiency The work load at heart rate 130 W_{130} was

calculated by intra or slight extrapolation With decreasing W_{max}, W_{130} became successively smaller The average heart volume or coronary angiographic picture did not change until at very low values of W_{max} Therefore, W_{max} and W_{130} or similar measures seem best suited to allow quantitative evaluation of myocardial function in the early and middle stages of coronary heart disease

No noteworthy heart rate and electrocardiographic reactions to the orthostatic test were observed

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pathophysiology of VA it was thought of interest to examine whether the changes observed in VA were affected by various drugs which influence the autonomic nervous system In a first study the ganglionic blocking agent chlorisondamine (Ecolid, Ciba trade mark) (32) was tested

Material

Among the patients who were sent to the Department of clinical physiology for examination of heart function and exhibited one or several of the symptoms and signs which are common in neurocirculatory asthenia and in vasoregulatory asthenia the following eleven patients have been studied

1) Two female patients (I A. and A M) with a low physical working capacity in relation to heart volume and sympathicotonic ECG-changes When later subjected to physical training, their physical working capacity became normal and the ECG changes decreased or disappeared This effect of physical training indicated a functional disturbance and sustained the diagnosis of vasoregulatory asthenia (20, 23)

2) Nine patients (six females and three males) with a low physical working capacity in relation to total amount of hemoglobin (THb) and heart volume (HV) and with sympathicotonic ECG-changes common in vasoregulatory asthenia were examined Five of these patients were examined with heart catheterization and the diagnosis confirmed

One of the last mentioned patients (E L) had an arterial hypertension of a slight degree She was therefore treated separately and not included in the comparisons between the VA patients and the controls

Some general anthropometric data of the patients are given in Table I Table II shows some of the patients symptoms which partially or entirely caused the patients to be sent to the laboratory Several of the patients had been examined (ECG at rest and during exercise) repeatedly at the laboratory during the last few years

Most of these VA patients were sent by local practitioners or came from the psychiatric clinic Common preliminary diagnoses were neurocirculatory asthenia, myocarditis postinfectious asthenia and symptomatic diagnosis such as precordial pain or dyspnoea

The controls were nine healthy volunteers and three patients (two males and one female) The three patients were sent from various hospitals for examination of heart function because of a systolic murmur over the pulmonary area or suspected radiological abnormalities of the heart One female volunteer and the three patients were examined with heart catheterization (control group I) The hemodynamic study did not indicate any heart disease and the systolic murmurs were regarded as accentuated physiological murmurs The female volunteer had a slightly low hemoglobin concentration. The remaining 8 control subjects (one male and seven females) formed control group II

The controls were examined with the same methods as the VA patients Some anthropometric data of the control subjects appear in Table I

From the Department of Clinical Physiology, University of Umeå, Umeå, Sweden

The Effect of a Ganglionic Blocking Agent (Chlorisondamine) on Electrocardiogram, Physical Working Capacity and Hemodynamics in Patients with Vasoregulatory Asthenia

By

O. Arvedson, C. Furberg and H. Linderholm

Vasoregulatory asthenia (VA) has been described (22) as a pathophysiological syndrome with the following characteristics. A low physical working capacity in spite of normal stroke volume of the heart, a high cardiac output in relation to the oxygen uptake and a low arterio-venous oxygen difference at rest as well as during exercise, and normal blood pressures. The hyperkinetic circulation is probably due to an inadequate regulation of the peripheral blood flow. Patients with VA show a variable tachycardia at rest and an unusually high pulse rate in the upright position. Typical are also ECG-changes often described as sympathicotonic (23). The VA syndrome may be present in patients with symptoms of neurocirculatory asthenia (Da Costa's syndrome) but may also occur in patients without such symptoms (21).

It has been suggested that an increased sympathetic tone might be a pathophysiological mechanism in the VA syndrome (23). ECG-changes similar to those found in VA can be produced by intravenous infusion of epinephrine in healthy men (6) and similar sympathicotonic ECG-changes have also been observed in various experimental and clinical states with increased sympathetic tone (5, 8, 29, 42, 43, 48 and others).

A hyperkinetic circulation, although less pronounced than in the patients with VA, is found in anxious, stressed patients in the resting state (19), i.e. in a condition assumed to be associated with increased sympathetic tone. Adrenaline in small doses also causes vasodilatation and a hyperkinetic circulation with an increase in cardiac output and a decrease in the arterio-venous oxygen difference at rest (14, 46).

Animal experiments suggest that a hyperkinetic condition may be caused by increased nervous stimulation mediated by hypothalamic centers either to the adrenals, resulting in an increased secretion of adrenaline (4, 16), or via the sympathetic vasodilator nerves to skeletal muscles (7).

Assuming an increased sympathetic tone to be an essential factor in the

pathophysiology of VA it was thought of interest to examine whether the changes observed in VA were affected by various drugs which influence the autonomic nervous system. In a first study the ganglionic blocking agent chlorisondamine (Ecolid, Ciba trade mark) (32) was tested.

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Assuming an increased sympathetic tone to be an essential factor in the

Table II Symptoms of V A patients

Subjects	Precordial pain	Palpitations	Breathlessness	Peripheral symptoms[1]	Anxiety
I V.		+	+	+	+
A M	+	+			
A K		+			+
A E	+	+	+		
G R		+			
G S				+	
S V		+	+		+
H K	+	+		+	+
K E.K	+		+		+
T W			+		
E.L.	+	+	+		

1 = Include dizziness cold hands and feet and headache

orthostatic test was recorded with standard leads I—III, unipolar extremity leads, aVR, aVL, aVF and precordial leads CR_1 CR CR_4, CR_5 CR_7 The ECG was also recorded during and after a work test. During work performed in the sitting position, the indifferent lead was on the forehand and only precordial leads were used (CH CH_4 CH , and CH_7) The sympathicotonic ECG-changes were classified according to a 7 grade scale (23)

An electrically braked bicycle ergometer (24) was used to determine the *physical working capacity* according to Sjostrand and Wahlund (40 47) Females usually started working at a load of 200 kpm/min and males at 300 kpm/min The work load was then increased stepwise every 6th minute by 200 kpm/min for females and 300 kpm/min for males The patients as well as the controls continued cycling until they found the work too heavy The physical working capacity at a pulse rate of 170 beats/min (PWC_{170}) was estimated, using the approximately linear relationship between the work load and the pulse rate after 6 minutes' work on each load By inter or extrapolation, the work load in kpm/min corresponding to a heart rate of 170 beats/min was obtained The predicted normal value for PWC_{170} of an individual was obtained from the regression equation Predicted $PWC_{170} = 1.60\ THb - 141$ (21)

The maximal work intensity at a relative steady state PWC_{max}, was taken as the heaviest load at which the subject actually worked for 6 minutes If the subject was able to work for 2—4 minutes on a heavier load half of the increase in work load was added

The work tests before and during the action of chlorisondamine were usually performed within two days

Arterial blood pressure was measured indirectly according to Riva Rocci (3) *Total amount of hemoglobin* (THb) was determined according to the al

TABLE I Some anthropometric data from VA patients and controls The VA patient E L had arterial hypertension

		Age years	Height cm	Weight kg	Hb conc g/100 ml	THb g	HV ml	HV/THb	Predicted PWC_{170}
VA patients									
I A	F	37	161	57	12 4	—	490	—	—
A M	F	26	163	53	11 5	—	510	—	—
A K	F	21	165	53	9 9	490	470	0 96	640
V E	F	37	163	65	12 5	490	540	1 10	640
G R	F	33	158	52	10 5	445	450	1 01	570
G S	M	20	172	73	14 6	635	640	1 01	875
S N	F	36	168	68	12 5	520	620	1 19	690
H K	F	29	158	58	11 9	505	470	0 92	665
K E K	M	54	167	70	12 4	620	660	1 06	850
T W	M	41	172	67	11 9	725	670	0 92	1020
Mean		33	165	62	12 0	554	552	1 02	745
E L	F	44	163	75	11 9	565	580	1 03	765
Controls									
Group I									
n		4	4	4	4	4	4	4	4
Mean		20	171	60	12 5	561	639	1 15	755
Range		17 22	158-181	43 71	10 2 14 5	385-720	475 835	0 95 1 26	475 1010
Group II									
n		8	8	8	8	8	7	7	8
Mean		20	167	59	11 7	498	576	1 28	655
Range		17 23	160-178	50 67	10 9 13 0	410 600	490 655	0 99 1 51	575 820

List of symbols
Hb conc = hemoglobin concentration
THb = total amount of hemoglobin
HV = heart volume
PWC_{170} = physical working capacity at pulse rate 170/min
Predicted PWC_{170} = 1 60 × THb—141
n = number of patients examined
M = male
F = female

Methods

The VA patients and the controls were examined before and 60—90 minutes after a subcutaneous injection of 0 1 mg chlorisondamine per kg of body weight Preliminary studies had shown that after such a dose the maximal orthostatic effects on pulse rate and blood pressure appeared between 1 and 1½ hours after the injection (11)

A ganglionic blocking effect of the drug was indicated by at least two of the following signs

1 Blurring of vision and dryness in the mouth

2 A lower arterial blood pressure during an orthostatic test than before the administration of chlorisondamine

3 A lower arterial blood pressure at rest in the supine position than before the drug was given

The *ECG* at rest and during an

TABLE II Symptoms of VA patients

Subjects	Precordial pain	Palpitations	Breathlessness	Peripheral symptoms[1]	Anxiety
I A		+	+	+	+
A M	+	+			
A K		+			+
A E	+	+	+		
G R		+			
G S				+	
S V		+	+		+
H K	+	+		+	+
K E K	+		+		+
T W			+		
E L	+	+	+		

[1] = Include dizziness cold hands and feet and headache

orthostatic test was recorded with standard leads I—III unipolar extremity leads, aVR aVL, aVF and precordial leads CR_1, CR , CR_4 CR, CR- The ECG was also recorded during and after a work test During work performed in the sitting position the indifferent lead was on the forehand and only precordial leads were used (CH- CH_4 CH , and CH_7) The sympathicotonic ECG changes were classified according to a 4 grade scale (23)

An electrically braked bicycle ergometer (24) was used to determine the *physical working capacity* according to Sjostrand and Wahlund (40, 47) Females usually started working at a load of 200 kpm/min and males at 300 kpm/min The work load was then increased stepwise every 6th minute by 200 kpm/min for females and 300 kpm/min for males The patients as well as the controls continued cycling until they found the work too heavy The physical working capacity at a pulse rate of 170 beats/min (PWC_{170}) was estimated using the approximately linear relationship between the work load and the pulse rate after 6 minutes work on each load By inter- or extrapolation the work load in kpm/min corresponding to a heart rate of 170 beats/min was obtained The predicted normal value for PWC_{170} of an individual was obtained from the regression equation Predicted PWC_{170} = 1 60 THb — 141 (21)

The maximal work intensity at a relative steady state, PWC_{max}, was taken as the heaviest load at which the subject actually worked for 6 minutes If the subject was able to work for 2—4 minutes on a heavier load half of the increase in work load was added

The work tests before and during the action of chlorisondamine were usually performed within two days

Arterial blood pressure was measured indirectly according to Riva Rocci (3) *Total amount of hemoglobin* (THb) was determined according to the al

veolar CO method, with slight modification of the original method (22, 41). Smaller amounts of CO were added to the rebreathing system and the CO analysis were made according to Andersson and Dahlstrom (2). The blood volume was calculated from the THb and the hemoglobin concentration of finger blood.

Heart volume (HV) was determined in the prone position (27, 28), with a distance of 125 cm between the focus and the film. The calculations were made according to Jonsell's method (26, 30).

Right heart catheterization and hemodynamic studies at rest and during exercise were performed in principle as described earlier by Holmgren et al. (22). The patients were studied at rest and in the supine position during exercise. A double-lumen heart catheter was placed with its tip in the pulmonary artery. A polyethylene or tephlone catheter was introduced into the brachial artery by percutaneous technique (36). Left to right and right to left intracardiac shunts of significance were excluded. Blood pressures were recorded on an Elema Klinik ECG apparatus using the Elema strain gauge mechano electrial transducer. The mid axillary line was taken as the zero pressure level.

Cardiac output was determined according to the direct Fick principle. Oxygen uptake was measured using the Douglas bag technique. During exercise the collection of expired air started after 4 minutes of exercise on each work load. Samples of arterial and mixed venous blood were drawn simultaneously during the collection of expired air.

A first study at rest and during exercise was performed in the morning, two to three hours after 0.1 g of pento-barbital sodium and 0.4 g chinidin were given orally. About 15 minutes after completion of this examination, a subcutaneous injection of 0.1 mg chlorisondamine per kg body weight was given. Ninety minutes later the examination was repeated and usually completed within 30 minutes.

Two patients (S.N. and H.K.) were examined before and during the action of chlorisondamine on two occasions: before and in connection with the heart catheterization. In the rest of the subjects who were examined with heart catheterization, the effect of chlorisondamine was investigated only in connection with the heart catheterization study.

Blood and gas analysis. The micro Scholander method (35) was used for the determination of O_2 and CO_2 of expired air, the volume of which was measured in a gasometer.

The O_2 content and O_2 saturation of blood was measured with the van Slyke manometric method (44) or with a spectrophotometric method (25). The hemoglobin concentration was measured as oxyhemoglobin (45).

Most of the statistical calculations were made according to Snedecor (39). The differences between the degree of ECG changes were tested with the Wilcoxon matched pairs signed ranks test (37).

Results

ECG and physical working capacity

Typical *ECG-changes* in a VA patient

TABLE III Ecg changes in VA patients and the effect of chlorisondamine

Subjects	Rest		Standing		Work	
	0	*Chl*	*0*	*Chl*	*0*	*Chl*
I A	1	0	2	1	0	0
A M	1	0	2	2	1	1
A K	4	0	4	1	3	1
A E	3	0	3	1	4	3
G R	1	0	3	0	2	1
G S	4	0	4	0	4	2
S N	2	0	4	1	3	1
H K	0	0	1	0	1	0
Median	1.5	0	3 0	1 0	2 5	1 0

0 = without chlorisondamine
Chl = with chlorisondamine
Ecg changes were classified according to a four grade scale 0 being no change and grades 1—4 being successively more pronounced abnormal changes of ST and T (23)

are shown in Fig 1 The sympathicotonic ECG changes decreased after chlorisondamine Table III shows the degree and distribution of the ECG changes at rest, during an orthostatic test and at exercise, before and after chlorisondamine With chlorisondamine the ECG changes are statistically significantly smaller at rest in the supine as well as in the standing position ($P<0\ 01$) and during exercise ($P<0\ 05$) In the controls, chlorisondamine caused insignificant ECG changes as compared to those in the VA cases

The *pulse rate* at rest in the recumbent position and during the orthostatic test was high in the VA patients and in contrast to the controls it decreased after chlorisondamine thereby approaching the pulse rate of the controls after ganglionic blockade (Table IV) The de

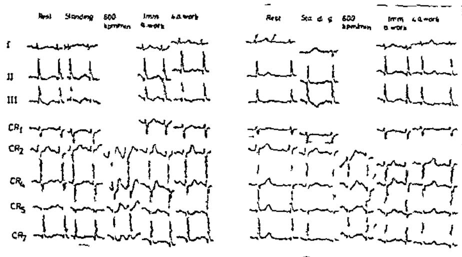


Fig 1 The effect of chlorisondamine on ECG *at rest during* an orthostatic test, and in connection with exercise in a patient (A K) with vasoregulatory asthenia Left before right 60—90 *minutes after* a subcutaneous injection of 0 1 mg chlorisondamine per kg body weight

TABLE IV Pulse rate and blood pressure at rest (recumbent) and standing upright, in patients with VA and in controls before and during the action of chlorisondamine (0 1 mg/kg body weight)

	Rest				Standing			
	Pulse rate beats/min		Syst blood pressure mm Hg		Pulse rate beats/min		Syst blood pressure mm Hg	
Subjects	0	Chl	0	Chl	0	Chl	0	Chl
VA patients (n=8)								
I A	104	90	125	120	145	91	120	100
A M	110	107	130	130	136	100	135	100
A K	140	94	160	130	142	101	140	135
A E	108	97	150	140	140	99	145	65
G R	89	106	125	110	110	107	130	70
G S	109	98	160	140	108	118	160	115
S N	90	85	130	125	122	106	130	110
H K	85	97	145	130	114	120	135	110
Mean	104	97	140	128	127	105	136	101
S D	±17 5	±7 4	±15 0	±10 0	±15 3	±9 8	±11 9	±23.2
Controls (Group II) (n=8)								
Mean	77	85	128	122	94	107	126	109
S D	±10 2	±7 8	±10 3	±15 8	±11 4	±8 2	±12 7	±22 3

0 = without chlorisondamine
Chl = with chlorisondamine

crease in arterial blood pressure after chlorisondamine at rest in the recumbent and in the standing position was similar in VA patients and control subjects

The *physical working capacity*, PWC_{170}, tested without chlorisondamine, was rather variable in the VA patients during the investigation (Table V and VI) It tended to increase during the course of repeated work tests, which were performed in rather close succession by some of the VA patients This was presumably an effect of physical training (20)

The physical working capacity, PWC_{170}, evaluated from a work test in the sitting position, was low in the VA patients During the action of chlorisondamine it was considerably higher In the supine position, in connection with heart catheterization, the PWC_{170} also increased during the action of the drug, but less (Table VI) If compared to the PWC_{170} obtained at the very first work test (no I) this increase, however, was considerably higher in each patient This may be due to the fact that the subjects submitted to heart catheterization were prepared for this examination by repeated work tests which evidently had an effect of physical training The dif-

ference between the $PWC_{1\,0}$ obtained from the work test No I and the work test in the supine position without chlorisondamine during the heart catheterization is probably not due to orthostatic effects because it has been shown earlier (22) that there is no appreciable difference between the $PWC_{1\,0}$ in the sitting and supine position in VA patients In contrast to the VA patients neither training (the repeated work tests) nor the drug changed $PWC_{1\,0}$ significantly in the healthy control subjects (Table V VI)

Under the influence of chlorisondamine the pulse rate during work at an equal load decreased considerably in the VA patients but not in the controls

The *maximum work intensity* at a relative steady state PWC_{max} seemed to increase in the VA patients but rather decreased in the controls after chlorisondamine *The maximum pulse rate recorded* at the exercise tests decreased after chlorisondamine about as much in VA patients as in controls (Table V)

The ratios $PWC_{1\,0}$/THb and $PWC_{1\,0}$/HV were low in the VA patients but after chlorisondamine they increased into the range of the controls In the healthy controls chlorisondamine did not significantly influence these ratios (Table V and VI)

Some differences between the effects of chlorisondamine on VA patients and on *controls are summarized in* Table VII

Hemodynamic studies

Some of the effects of chlorisondamine on the hemodynamic conditions as studied during heart catherization are summarized in Table VIII and Figs 2 and 3

The *pulse rate* at rest and during exercise decreased after chlorisondamine in the VA cases but increased slightly in the controls This was in agreement with the findings before catheterization

The *blood pressures in the lesser circulation* at rest and during exercise were normal both in VA patients and controls and seemed to decrease slightly when chlorisondamine was given

The *systemic arterial blood pressures* at rest and during exercise were normal in the controls and all VA patients but one (E L) before chlorisondamine was given The blood pressure in the hypertensive patient (E L) was normalized by chlorisondamine The systolic pressure decreased in all cases after chlorisondamine The mean blood pressure decreased *in all the VA patients examined* at rest and during exercise, but was largely *unchanged in the controls* (Table VIII)

The *oxygen uptake* at rest decreased after chlorisondamine at an average by 6 per cent both in VA patients and controls During exercise at comparable work loads the oxygen uptake seemed to be largely unaffected by chlorisondamine in VA patients and controls (Table VIII)

The *arterial oxygen saturation* was normal at rest and during exercise in all VA cases and controls before and after chlorisondamine

The *arterio-venous oxygen difference* at rest and during exercise at identical work loads increased after chlorisondamine in both groups The *increase* seemed to be greater in the VA cases than in the controls

TABLE IV Pulse rate and blood pressure at rest (recumbent) and standing upright in patients with VA and in controls before and during the action of chlorisondamine (0,1 mg/kg body weight)

	Rest				Standing			
	Pulse rate beats/min		Syst blood pressure mm Hg		Pulse rate beats/min		Syst blood pressure mm Hg	
Subjects	0	Chl	0	Chl	0	Chl	0	Chl
VA patients (n=8)								
I A	104	90	125	120	145	91	120	100
A M	110	107	130	130	136	100	135	100
A K	140	94	160	130	142	101	140	135
A E	108	97	150	140	140	99	145	65
G R	89	106	125	110	110	107	130	70
G S	109	98	160	140	108	118	160	115
S N	90	85	130	125	122	106	130	110
H K	85	97	145	130	114	120	135	110
Mean	104	97	140	128	127	105	136	101
S D	±17 5	±7 4	±15 0	±10 0	±15 3	±9 8	±11 9	±23 2
Controls (Group II) (n=8)								
Mean	77	85	128	122	94	107	126	109
S D	±10 2	±7 8	±10 3	±15 8	±11 4	±8 2	±12 7	±22 3

0 = without chlorisondamine
Chl = with chlorisondamine

crease in arterial blood pressure *after* chlorisondamine at rest in the recumbent and in *the standing* position was similar in VA patients and control subjects

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ference between the $PWC_{1\,0}$ obtained from the work test No I and the work test in the supine position without chlorisondamine during the heart catheterization is probably not due to orthostatic effects, because it has been shown earlier (22) that there is no appreciable difference between the $PWC_{1\,0}$ in the sitting and supine position in VA patients In contrast to the VA patients neither training (the repeated work tests) nor the drug changed $PWC_{1\,0}$ *significantly in the healthy control subjects* (Table V, VI)

Under the influence of chlorisondamine the pulse rate during work at an equal load decreased considerably in the VA patients but not in the controls

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The ratios $PWC_{1\,0}$/THb and $PWC_{1\,0}$/HV were low in the VA patients but after chlorisondamine they increased into the range of the controls In the healthy controls chlorisondamine did not significantly influence these ratios (Table V and VI)

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The *systemic arterial blood pressures* at rest and during exercise were normal in the controls and all VA patients but one (E L), before chlorisondamine was given The blood pressure in the hypertensive patient (E L) was normalized by chlorisondamine The systolic pressure decreased in all cases after chlorisondamine The mean blood pressure decreased in all the VA patients examined at rest and during exercise but was largely unchanged in the controls (Table VIII)

The *oxygen uptake* at rest decreased after chlorisondamine at an average by 6 per cent both in VA patients and controls During exercise at comparable work loads the oxygen uptake seemed to be largely unaffected by chlorisondamine in VA patients and controls (Table VIII)

The *arterial oxygen saturation* was normal at rest and during exercise in all VA cases and controls before and after chlorisondamine

The *arterio venous oxygen difference* at rest and during exercise at identical work loads increased after chlorisondamine in both groups The increase seemed to be greater in the VA cases than in the controls

TABLE V Physical working capacity (PWC_{170}), pulse rate at a given load on the bicycle ergom a relatively steady state, PWC_{max}, maximal pulse rate, and the PWC_{170} in rela- heart volume (HV) in VA patients and in controls before and during the action of Work in sitting position

Subjects	PWC1-0 kpm/min		Pulse rate at equal load beats/min		PWCmax kpm/min		Max pulse rate beats/min	
	0	Chl	0	Chl	0	Chl	0	Chl
VA patients (n=8)								
I A	400	600	170	152	500	600	179	170
A M	345	780	177	149	500	500	187	149
A K	400	800	180	154	600	600	180	160
A E	300	600	154	120	300	600	173	170
G R	200	550	189	150	400	400	189	151
G S	540	740	178	155	750	750	198	182
S N	350	720	145	105	300	500	175	145
H K	420	620	166	139	500	600	187	167
Mean	369	676	170	140	481	569	183	162
S D	±98 6	±94 7	±14,5	±18,4	±151	±103	±8,3	±12,7
Controls (Group II, n=8)								
Mean	697	752	171	164	850	787	187	169
S D	±123	±160	±15 9	±12,2	±180	±190	±6 0	±10 4

TABLE VI Physical working capacity (PWC_{170}) determined from the first work test in the sitting position (no I) and in the supine position before (0) and during (Chl) the action of chlorisondamine in connection with the heart catheter study The ratios PWC_{170}/THb and PWC_{170}/HV are given for the same experimental conditions in 5 VA cases and the controls The VA patient E L, who had arterial hypertension is treated separately

case	PWC1 0			PWC1 0/THb			PWC1-0/HV		
	sitting	supine (cath)		sitting	supine (cath)		sitting	supine (cath)	
	no I	0	Chl	no I	0	Chl	no I	0	Chl
VA patients									
H K	250	340	420	0 49	0 67	0 82	0 53	0 72	0 89
S N	360	500	800	0 69	0 96	1 54	0 58	0 81	1 29
K E K	400	570	700	0 65	0 92	1 13	0 61	0 86	1 06
T W	580	580	750	0 80	0 80	1 03	0 87	0 87	1 12
Mean	398	498	668	0 66	0 84	1 13	0 65	0 82	1 09
E L	150	400	850	0 27	0 71	1 50	0 69	0 67	1 47
Controls (Group I)									
n	4	4	4	—	4	4	—	4	4
Mean	755	698	653	—	1 30	1 19	—	1 14	1 04
Range	540 880	550 890	550-810		1 22-1 46	1 06-1 43		0 96 1 35	0 84 1 18

e er maximal phys cal work ng capac ty at tion to to al amount of hemoglobin (THb) and chlo sondam ne (0 1 mg/kg body we ght)

$PWC_{1\,0}$/THb		$PWC_{1\,0}$ HV	
0	Chl	0	Chl
—	—	0 80	1 22
—	—	0 68	1 53
0 82	1 63	0 85	1 70
0 51	1 22	0 46	1 11
0 45	1 00	0 44	1 22
0 85	1 17	0 84	1 16
0 67	1 38	0 56	1 16
0 82	1 22	0 89	1 32
0 69	1 27	0 69	1 30
0 17	0 21	±0 18	±0 21
1 40	1 51	1 20	1 29
±0 20	±0 29	±0 21	±0 29

Cardiac output decreased after chlorisondamine in the VA patients at rest and during exercise at a high work load while in the controls the decrease was far less pronounced yet significant at rest The decrease in mean arterial pressure at rest seemed to be mainly due to a decrease in cardiac output while during work a decrease in both cardiac output and peripheral vascular resistance seemed to occur in the VA group (Table VIII)

The stroke volume at rest seemed to decrease in both groups by chlorisondamine while the stroke volume during exercise was largely unchanged (Table VIII)

Before chlorisondamine was given the *oxygen saturation of mixed venous blood* was higher and the *arterio venous oxygen difference* lower in relation to pulse rate in the VA patients than in

TABLE VII The effect of chlorisondamine expressed as the d fference between the results w th and w thout chlorisondam ne (Chl 0) D is the mean d fference w thin VA pat ents D_2 w th n con rols The d fference between the effect of chlorisondam ne n the two groups is represented by the d ff (D —D)

	VA pat ents		Cont ols		D ff
	n	D	n	D_2	(D D_2)
At res up ne					
Pul e ra e beats m n	10	— 8 2	12	+ 8 7	—16 9
Sys ol c blood p essure mm Hg	8	—12 5	8	— 5 6	— 6 9
Stand ng					
Pulse ra e beats m n	8	21 9*	8	+13 1	—35 0**
Sys c blood pressu e mm Hg	8	—36 3**	8	—16 9	—19 4
Wo k test					
PWC kpn m n	10	+276***	12	+22	+254***
Pulse a equal load beats m n	8	29 4***	8	— 6 9	—22 5***
PWC_{max} kpm m n	8	+83 5	8	—62.5	+159*
Max pulse ra e beats m n	8	—21 8**	8	—17 8***	— 4 0
PWC THb	8	+ 0 49***	12	+ 0 03	+ 0 46***
PWC HV	10	+ 0 53***	11	+ 0 02	+ 0 51***

* P 0 05 ** = P 0 01 *** = P<0 001 where P is the probability that the d fference is caused by random fa tors

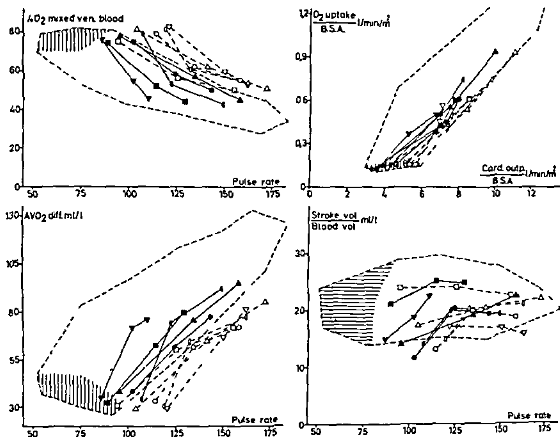


Fig 2 The effect of chlorisondamine on the O_2 saturation of mixed venous blood and the arterio-venous oxygen difference in relation to pulse rate (beats/min) the O_2 uptake in relation to cardiac index and the stroke volume divided by blood volume at rest and during exercise in five patients with vasoregulatory asthenia The symbols of the individual cases are as follows S N □ H K ○, K E K ◘, T W △ and E L ▽ The thin broken lines indicate the normal range of variation and the hatched areas the normal variation in the resting state (22) The unfilled symbols connected with broken lines denote results before chlorisondamine, the filled symbols connected with full lines indicate conditions during the action of chlorisondamine under otherwise comparable conditions e g equal work loads

the controls (Figs 2 and 3) The cardiac index in relation to oxygen uptake per body surface area was also higher on an average in the VA patients than in the controls The VA patients were, however, somewhat less hyperkinetic than the majority of the VA patients described by Holmgren et al (22) During the action of chlorisondamine the oxygen saturation of mixed venous blood and the arteriovenous oxygen difference in relation to the oxygen uptake per body surface area approached the relationship found in the controls

The changes induced by chlorisondamine on the relationship between pulse rate and a given value of mixed venous blood oxygen saturation (60 per cent) and between cardiac index and a given oxygen uptake (0 6 l/min) were statistically significant in the VA cases while the differences were insignificant in the controls

There was also a statistically signifi

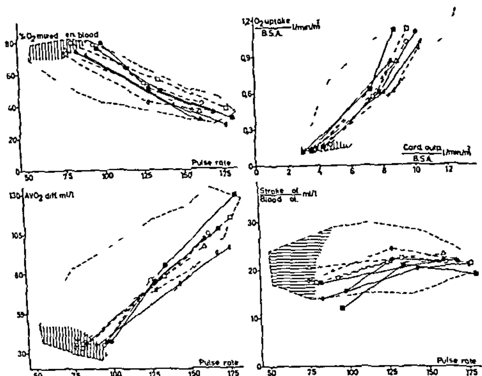


Fig 3 The same relationships as in Fig 2 for the four controls The symbols of the individual cases are as follows G.A △ A W ○ B B □ and L.F ○ Range of normal variation and the filled and unfilled symbols have the same meaning as in Fig 2

cant difference between the response of the VA patients and that of the controls to chlorisondamine with regard to the last two mentioned relationships as well as to the cardiac index in relation to oxygen uptake (Table VIII)

Discussion

Effect of chlorisondamine on the VA syndrome The results show that in patients with the vasoregulatory asthenia syndrome ganglionic blockade with chlorisondamine more or less completely abolished the abnormalities in ECG orthostatic pulse reaction physical working capacity and hemodynamic conditions, typical of the VA syndrome In healthy control subjects, chlorisondamine had only an insignificant effect on the ECG and the reactions and conditions markedly influenced in the VA patients.

During the action of chlorisondamine the PWC_{170} of the VA patients increased about 80 % and approached the PWC_{170} predicted from the THb, while in the controls there was an insignificant

TABLE VIII Means of some hemodynamic variables and their *percentage change* ($D\%$) during the action of chlorisondamine in 4 VA patients and 4 *controls* (*Group I*) The per centagn change for each individual subject was based on results from equal work loads before and during *the action of the* drug Symbols as in Table VII

	VA			Controls			$D_1 - D_2$
	n	0	D_1 %	n	0	D_2 %	
Mean arterial blood pressure, mm Hg							
At rest	3	96	—10	3	91	— 2	— 8
During exercise moderate pulse rate	3	107	—11	3	95	— 1	— 9
During exercise, high pulse rate	3	118	—15	3	107	— 3	—12
Oxygen uptake, ml/min							
At rest	4	260	— 6**	4	236	— 6**	± 0
During exercise, moderate pulse rate	4	822	+ 3	3	1069	+ 2	+ 1
During exercise, high pulse rate	4	1256	± 0	4	1679	+ 4	— 4
Arterio venous oxygen difference ml/l							
At rest	4	31	+16	4	35	+ 5	+11
During exercise, moderate pulse rate	4	63	+ 9	3	75	+ 5	+ 5
During exercise high pulse rate	4	76	+12*	4	102	+ 6	+ 6
Cardiac output, l/min							
At rest	4	8,5	—18*	4	6 8	—10*	— 8
During exercise moderate pulse rate	4	13,1	— 6	3	14 3	— 3	— 4
During exercise, high pulse rate	4	16 3	—11*	4	16 5	— 1	—10
Peripheral vascular resistance mm Hg l/min							
At rest	3	10 5	+ 9	3	12 8	+11	— 2
During exercise, moderate pulse rate	3	7,8	— 3	3	6 6	+ 2	— 5
During exercise, high pulse rate	3	6,9	— 4	3	6 0	— 2	— 3
Stroke volume, ml							
At rest	4	79	—10	4	83	—20**	+10
During exercise moderate pulse rate	4	99	+ 1	3	112	— 7	+ 8
During exercise high pulse rate	4	100	± 0	4	99	— 4	+ 4
Pulse rate at an oxygen saturation of 60 % in mixed venous blood beats/min	4	135	—12***	4	112	+ 2	—14**
Pulse rate at an arterio-venous oxygen difference of 55 ml/l beats/min	4	127	—10**	4	106	+ 6*	— 16***
Cardiac index at an oxygen uptake of 0,6 l/min m² BSA	4	8 6	—11*	4	7,7	— 3	— 8*

change in $PWC_{1\ 0}$ Also the maximal work intensity that the VA patients were able to perform in a relatively steady state (PWC_{max}) seemed to increase with chlorisondamine particularly in some individual patients while in the controls it rather decreased

The maximal pulse rate decreased during the action of chlorisondamine This may explain why PWC_{max} did not increase as much as $PWC_{1\ 0}$

The results from the heart catheterization examinations show that the effect of the drug on the physical working capacity was mainly due to the fact that the drug caused a higher degree of utilization of the blood oxygen in the tissues and consequently a higher arterio-venous oxygen difference at a given heart rate as well as at a given work load The VA patients however, had at the time of the heart catheterization a comparatively mild degree of VA as compared with those studied by Holmgren et al (22) As already mentioned this might to some extent be due to the effect of physical training from several work tests before the examination (20 21) The patient E L who had functional changes typical of VA but also a slight arterial hypertension behaved in most respects in a similar way as the other VA patients

Mechanism of chlorisondamine action Chlorisondamine causes a partial or complete blockade of ganglia of the sympathetic and parasympathetic division of the autonomic nervous system (18 32) The normalizing effect of the drug on patients with VA sustains the idea that an imbalance in the autonomic system is a pathophysiological mechanism in the VA syndrome This autonomic imbalance most likely consists of a relative increase in sympathetic tone Several signs and symptoms in VA patients are consistent with such an increase in sympathetic tone

Chlorisondamine does not block the effect of injected adrenaline or acetylcholine (17, 32) but presumably it blocks the release of adrenaline from the adrenal gland in response to splanchnic nerve stimulation (17) It seems therefore likely that the mechanism of action of the drug in VA patients is a blockade of abnormal efferent impulses from the central nervous system to the peripheral vessels and/or to the adrenal medulla

Abnormal nervous impulses have earlier been suggested as a possible cause of VA (22) and these impulses may be similar to those caused by stimulation of a hypothalamic area in experimental animals (1, 7 16) Such a stimulation has been reported to cause vasodilatation of muscular vessels, tachycardia, increased cathecolamine secretion and probably increased cardiac output (9 16 33) as well as reactions of fear and rage in conscious animals (1) i e symptoms of an overactivity of the sympathoadrenal system.

It has not been shown that there is an increased secretion of adrenaline in VA So far the urinary excretion of cathecolamines seems to have been examined only in three VA patients (22) during night during rest in the morning and during an orthostatic test and a work test The values were relatively high for adrenaline excretion during rest in the morning but otherwise not remarkable Further investigations seem to

be required in order to decide if adrenaline is secreted in increased amounts in VA or not However, small amounts of adrenaline are known to induce a hemodynamic condition similar to the VA syndrome (14, 46) and if an increased adrenaline secretion were a main mechanism in VA one might expect an effect of ganglionic blocking agents by blocking impulses to the adrenal medulla (17)

If sympathetic nervous impulses to the heart and the peripheral vessels were a main mechanism in VA, abnormal sympathetic vasodilatator impulses to the muscle resistance vessels might be mainly responsible for the hyperkinetic circulation and vasodilatation, which has been found to be present in muscles of VA patients (12, 13) Such a concept does not fit with the observation that atropine, which is known to block the sympathetic vasodilatator impulses in animals (16), did not decrease the hyperkinetic blood flow through the muscles of VA patients (12, 13) However, the blocking effect of atropine might have been incomplete in experiments on man In spite of intraarterial injection (13) its effect might have been smaller than that obtained in experimental animals This belief is further sustained by the observation (15) that vasodilatation in the forearm muscles during emotional stress was not prevented by the intra arterial injection in the brachial artery of 0 3—1 5 mg atropine in human subjects but was almost abolished by nervous block

A reduction of the basal myogenic tone of the resistance vessels of voluntary muscles was proposed to be the main mechanism in VA (12, 13) In the light of our experiments this does not seem to be likely because chlorisondamine is not known to have any direct effect on smooth muscles (32)

The effect of chlorisondamine is of interest with regard to the fact that a number of different drugs have been tested clinically on patients with VA without much improvement in their condition (20) The favourable effect of physical training on patients with VA is, however, well established (20, 21)

Chlorisondamine for differentiation between 'functional' and 'organic" ECG-changes It has been clearly demonstrated in this study that chlorisondamine makes sympathetic ECG changes more or less completely disappear

We have observed that the effect of chlorisondamine on ECG changes in patients with coronary insufficiency or other organic heart diseases is usually insignificant, nor does the drug improve the physical working capacity in these patients Furthermore, chlorisondamine is not known to have a vasoconstrictor effect on the coronary vessels as do ergot derivatives (34), all these aspects might suggest its usefulness in the sometimes difficult task of differentiating between ECG-changes of functional and organic origin, particularly if the combined effect of chlorisondamine on the ECG and the physical working capacity is evaluated Other drugs, among them ergot derivatives (31), banthine, probanthine have been tried earlier in attempts to differentiate between functional ECG changes and those caused by coronary insufficiency and other organic heart disease A thorough review hereof has been d---

by Simonson in 1961 (38) who concluded, at that time, that there was not sufficient evidence for the diagnostic application of sympatholytic or vagolytic drugs Our results indicate that this conclusion should be revised Results similar to those seen after administration of chlorisondamine have later been obtained by use of propranolol, an adrenergic beta blocking agent (10)

While chlorisondamine may thus be a valuable diagnostic tool, it may give, on the other hand, some unwanted side effects such as transient blurring of vision mouth dryness and orthostatic hypotension A marked fall of blood pressure lasting for several hours has been observed in a few patients who are not included in this study It should therefore be used with some caution as the individual sensitivity seems to be rather variable

Summary

The effect of the ganglionic blocking agent chlorisondamine was studied in 11 patients with vasoregulatory asthenia (VA) and 12 healthy controls

In the VA patients chlorisondamine changed the sympathicotonic ECG the marked orthostatic pulse reaction the low physical working capacity and the hyperkinetic circulation all symptoms typical of the VA syndrome towards normal conditions In the healthy control subjects the drug had a rather insignificant effect on ECG physical working capacity and the hemodynamic conditions

The results sustain the idea that a relative increase in sympathetic tone may be a pathophysiological mechanism in the VA syndrome The mechanism of action of chlorisondamine is discussed as well as the possibilities to use chlorisondamine as a diagnostic aid in differentiating functional ECG-changes in VA from those due to coronary insufficiency or other organic heart diseases

Acknowledgement

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From the Laboratory of Clinical Physiology, Thoracic Clinics, Karolinska Sjukhuset Stockholm

The Instantaneous Effect of Aortic Pressure on Atrial Rate in Complete Atrioventricular Block

By

Sture Bevegård, Bengt Jonsson and Ingvar Karlof

In experimentally induced atrioventricular block in dogs, Erlanger and Blackman (4) found an atrial arrhythmia characterized by a long atrial cycle following a ventricular contraction and a successive shortening of the atrial cycles until the next ventricular contraction They stated that this phenomenon was probably due to rhythmical variations of vagal tone, which increases with each arterial pulse Subsequently this atrial arrhythmia also has been observed in patients (1, 5, 6, 8, 9, 12, 13, 14) The arrhythmia is abolished when anti cholinergic drugs are given (5, 13, 14) and most authors explain the phenomenon as a baroreceptor reflex with an increase of vagal tone with each aortic pressure pulse

During studies of the hemodynamic effects of artificial pacing in atrioventricular block, we observed an atrial arrhythmia in many patients Central aortic pressure was recorded in these cases, and it was possible to study the relationship between the systemic pressure and the atrial rate

Material

Sixteen patients with complete atrioventricular block were examined Their ages varied between 14 and 72 years More marked atrial arrhythmia was *present in only eight cases In patients* with a high atrial rate, the arrhythmia was less frequent than in patients with slow rate (see Table I)

Methods

The technique used for intra arterial catheterization and the pressure recording system was the same as previously reported from this laboratory (2) The tip of the catheter was placed in the subclavian artery The distances from the aortic valve to the tip of the catheter and to the carotid sinus were approximately equal The pressure was recorded together with an electrocardiographic lead giving easily defined P-waves

Results

The duration of the P—P interval is related to arterial pressure at the be-

TABLE I Age and average atrial rate in 8 cases with marked (A) and 8 cases with very slight (B) variation of the P P interval

Case No	Age Years	Average Atrial rate	Variation of P P interval csec
A			
1	20	81	12
2	24	75	11
3	30	86	19
4	37	80	18
5	38	92	18
6	41	68	21
7	62	65	14
8	63	79	16
B			
9	14	147	1
10	22	107	2
11	28	90	2
12	42	90	1
13	60	112	2
14	63	100	1
15	67	90	2
16	72	90	2

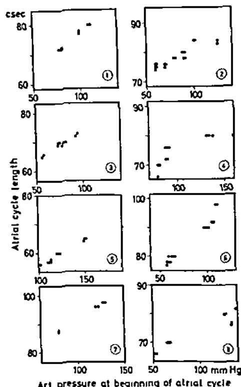


Fig 1 Atrial cycle length in relation to the arterial pressure at beginning of atrial cycle

ginning of the interval A linear correlation is found in all eight cases with atrial arrhythmia (Fig 1)

In order to determine the timing between arterial pressure pulse and the maximal and minimal P—P intervals, one patient was selected with no respiratory variation of the arterial pressure (Fig 2) The position of the P wave in the ventricular cardiac cycle as referred to *the arterial pressure pulse* (time axis on the abscissa) is related to the length of the following P—P interval (ordinate) The P—P intervals ($P_n \rightarrow P_{n+1}$) were measured from a long series of cardiac cycles with practically identical arterial pressure pulses The variation of the P—P intervals follows closely the variation in arterial pressure.

Discussion

The atrial arrhythmia in patients with complete atrioventricular block is not seen in patients with a very high atrial rate A high vagal tone seems to be a prerequisite for the arrhythmia The close correlation between the variation of the atrial rate and the systemic arterial pressure supports the earlier concepts of the importance of the baroreceptor reflex for the phenomenon

From the Laboratory of Clinical Physiology, Thoracic Clinics, Karolinska Sjukhuset, Stockholm

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Results

The duration of the P—P interval is related to arterial pressure at the be-

close conformity of the arterial pressure curve and the curve of varying P—P intervals (Fig 2) indicates that it is not the integrated pressure during the whole P—P interval which effects the atrial rate but the pressure during a much shorter interval The latent period, as defined above shows a marked variation Patients with a long P—P interval have a longer latent period than patients with a short P—P interval (Fig 3) The transmission time from the carotid sinus through the nerves and synapses would not differ that much If the vagal influence on the sinus rate is predominant during the period of repolarization and there is little or no effect during the last part of diastole, the latent period must be longer when the rate is slow since variation of the atrial cycle length mainly influences the diastolic period

Summary

Variation of the atrial rate with the systemic pressure pulse in complete atrioventricular block as first described by Erlanger and Blackman (4) was found in 8 of 16 cases studied By recording the intra arterial pressure it was possible to demonstrate a very close relation between the pressure variations in the carotid sinus and variation of the atrial rate This supports the earlier concepts that the phenomenon is caused by a baroreceptor reflex Our findings indicate that a rather short period in the middle of the P—P interval is most sensitive for the inhibitory effect of increased vagal tone on the atrial rate Probably the phase of repolarization in the sinus node is the sensitive part of the cycle

Acknowledgement

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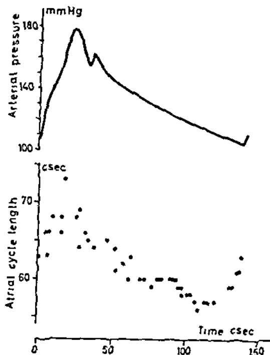


Fig 2 Time relation between arterial pressure and atrial cycle length in a case with no respiratory variation of arterial pressure (case no 5) *A* Arterial pressure in relation to time *B* Atrial cycle length ($P_n \rightarrow P_{n-1}$) from 55 measurements in relation to the interval between the beginning of systolic rise in arterial pressure (time = 0) and the beginning of the atrial cycle (P_n)

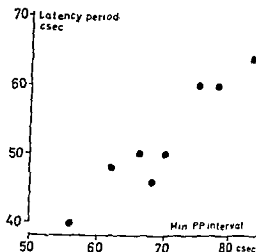


Fig 3 The latent period (defined as the shortest interval between the beginning of the systolic pressure rise in the carotid sinus and the end of that P P interval which can be prolonged by the pressure rise) in relation to the minimal P P interval

Probably it is caused by rhythmical variations of vagal tone, since the arrhythmia is abolished after vagal blocking both in animal experiments (7) and in patients (5 13 14) The latent period of this reflex from the change of pressure in the carotid sinus until the response in the sinus node, has been determined to 37 csec in dogs (1) and to 30—40 csec in two patients (6) In our case No 5 this latent period was calculated to 40 csec as follows The arterial pulse wave, which begins at time 137 csec on the x axis (see Fig 2), does not influence a P—P interval starting at time 120 and ending at 177 csec (40 csec after beginning of the arterial pulse wave) On the other hand, a P—P interval starting later in relation to the arterial pulse wave is influenced Thus the latent period is defined as the shortest interval between the beginning of the systolic pressure rise in the carotid sinus and the end of that P—P interval which can be prolonged by the pressure rise This latent period is much longer than the nerve conduction time (3) and is probably due mainly to low sensitivity of the sinus node to vagal stimulation during the late part of the atrial cycle Vagal stimulation causes a hyperpolarization of the cells in the sinus node and a slowing of the diastolic depolarization (11) It has also been observed that a vagal stimulation has its greatest effect on change in membrane potential during the phase of repolarization and its smallest effect at the end of the atrial cycle (10) These experimental studies explain the findings in our patients The

From he Labo a ory of Cl n cal Physiology Thorac c Clinics Karol nska sjukhuset S ockholm

Circulatory Effects of Increased Ventilation at Rest in Recumbent and Head up Tilted Position

By

Sture Bevegård Bengt Jonsson Ingvar Karlof and Hans Åstrom

Tl e ncrease n card ac output w th exerc se is bas cally l nked to metabolism as reflected n the oxygen uptake Several mechan sms contr bute to the attain ment and ma ntenance of a h gh card ac output such as the leg muscle pump (2) a d l e graded increase in venous tone (4 Tl e mportance of the ncrease in ent lat on for the increase in card ac ou put wi h exercise is not settled al though there are many reports on the hemodynam c effects of hypervent lat on (5 7 8 9 12 13 14 15 16 17) In most studies on man hyperyent lat on w th amb ent a r has been produced voluntarily resulting in hypocapnia or by adm nistrat on of 5—10 per cent CO to the n.p red air resulting n hyper capn a or a relat ve socapn a It can be concluded from these earl er stud es

Table I Some an h opome c da a of 12 pa ents w h normal c rcula on

Case no	Sex	Age years	Height, cm	Weigh kg	B S A. m	Vital capacity l	MVV l min
1	M	27	189	71	2 07	5 54	140
2	M	20	185	85	2 10	4 61	1 8
3	F	42	166	57	1 63	—	—
4	F	26	167	67	1 75	3 37	71
5	M	19	1 8	55	1 72	4 47	120
	F	21	165	49	1 54	3 23	70
7	M	18	175	59	1 74	3.50	95
8	M	19	171	60	1 72	4 96	98
9	M	21	172	55	1 66	4 8	131
10	M	17	179	65	1 84	4 62	110
11	M	19	1 8	67	1 86	5 67	116
1	F	19	166	63	1 0	3 0	64

MVV = Max n al olun ary entı a on at b ea h ng ra e 40 per m n

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From the Laboratory of Clinical Physiology, Thoracic Clinics, Karolinska Sjukhuset, Stockholm

Circulatory Effects of Increased Ventilation at Rest in Recumbent and Head-up Tilted Position

By

STURE BEVEGÅRD, BENGT JONSSON, INGVAR KARLÖF and HANS ÅSTRÖM

The increase in cardiac output with exercise is basically linked to metabolism as reflected in the oxygen uptake. Several mechanisms contribute to the attain ment and maintenance of a high cardiac output such as the leg muscle pump (2) and the graded increase in venous tone (4). The importance of the increase in ventilation for the increase in cardiac output with exercise is not settled although there are many reports on the hemodynamic effects of hyperventilation (5, 7, 8, 9, 12, 13, 14, 15, 16, 17). In most studies on man hyperventilation with ambient air has been produced voluntarily resulting in hypocapnia or by administration of 5—10 per cent CO_2 to the inspired air resulting in hyper capnia or a relative isocapnia. It can be concluded from these earlier studies

TABLE I. Some anthropometric data of 12 patients with normal circulation

Case no	Sex	Age years	Height cm	Weight kg	B S A m²	Vital capacity l	MVV, l/min
1	M	27	189	71	2 07	5 54	140
2	M	20	185	85	2 10	4 61	158
3	F	42	166	57	1 63	—	—
4	F	26	167	67	1 75	3 37	71
5	M	19	178	55	1 72	4 47	120
6	F	21	165	49	1 54	3 23	70
7	M	18	175	53	1 74	3 50	95
8	M	19	171	60	1 72	4 96	98
9	M	21	172	55	1 66	4 78	131
10	M	17	179	65	1 84	4 02	110
11	M	19	178	67	1 86	5 67	116
12	F	19	166	63	1 70	3 70	64

MVV = Maximal voluntary ventilation at breathing rate 40 per min

TABLE II C = control H = hyperventilation RV = right ventricle, PA = pulmonary artery, M = mean, D_I = initial diastolic D_E = end diastolic

Case no		Head up tilting degrees	Heart rate, beats/min	Ventilation l BTPS/min	Oxygen uptake ml STPD/min	Breathing rate/min	Tidal volume, l	AV O diff ml/l
1	C	0	74	9 2	302	15	0 61	34
	H	0	80	24 0	357	18	1 33	28
	C	60	73	7 6	253	17	0 45	36
	H	60	82	25 2	321	16	1 57	45
2	C	0	56	8 9	305	19	0 47	44
	H	0	81	16 0	308	14	1 14	38
	C	60	75	8 0	327	18	0 45	57
	H	60	104	20 4	349	14	1 46	50
3	C	0	79	7 5	243	14	0 54	31
	C	60	97	7 7	260	18	0 43	53
	H	0	84	21 8	245	19	1 15	26
	H	60	103	25 3	243	20	1 26	46
4	C	0	82	6 5	252	10	0 65	36
	C	60	92	6 6	237	10	0 66	62
	H	0	84	22 8	280	20	1 14	35
	H	60	100	25 3	311	18	1 41	53
5	C	0	68	9 4	282	30	0 31	47
	H	0	76	26 2	283	24	1 09	28
	C	60	125	13 7	242	32	0 43	73
	H	60	108	31 7	265	26	1 22	56
6	C	0	86	8 4	273	16	0 53	29
	H	0	102	25 6	282	20	1 28	25
	C	60	98	7 4	241	14	0 53	47
	H	60	119	28 1	305	20	1 40	42
7	C	0	89	11 4	280	20	0 57	28
	H	0	90	31 7	361	20	1 59	31
	C	60	111	18 1	280	26	0 69	51
	H	60	90	32 4	327	26	1 25	46
8	C	0	82	8 9	292	20	0 45	31
	C	60	97	8 2	282	18	0 45	50
	H	0	87	32 0	339	24	1 33	33
	H	60	104	30 7	332	22	1 39	43
9	C	0	78	8 4	268	20	0 42	34
	H	0	81	31 6	300	24	1 32	37
10	C	0	86	6 5	267	12	0 54	25
	H	0	101	18 5	365	16	1 16	30
11	C	0	83	8 5	290	24	0 36	33
	H	0	91	30 5	381	22	1 39	34
12	C	0	77	8 8	230	26	0 34	43
	H	0	88	19 7	300	16	1 23	42

PCV = pulmonary capillary venous Br A = brachial artery S = systolic D = diastolic,

Cardiac output l/min	Stroke volume ml	Pressures mm Hg RV S	RV D_I	RV D_E	PA S	PA D	PA M	PCV M	Br A S	Br A D	Br A M
8 9	120	26	3	7	24	12	17	11	116	81	96
12 6	157										
7 1	97	30	2	7	22	8	15		133	94	112
7 2	87	29	0	2	28	7	13		139	98	114
6 9	123	24	0	2	23	7	14	8	114	73	95
8 1	100				21	7	13		121	83	100
5 7	[illegible]				17	4	13		114	86	93
7 0	68				22	10	16		122	99	108
8 0	101	28	2	7	23	8	14	10	106	66	87
4 9	51	21		−1	12	2	6		106	70	86
9 6	114	27	−1	6	20	4	11				
5 3	52	24		−3	17	2	8		118	78	95
7 0	85	26	4	8	22	14	18	12	104	70	88
3 8	41	22	1	3	18	10	13		105	75	89
8 1	96	30	1	5	27	10	17		107	72	90
5 9	59	25	−1	1	20	7	13		116	78	94
6 0	89	25	5	9	19	11	15	11	120	70	93
9 9	130	24	1	5	19	9	14		115	72	93
3 3	27										
4 7	45										
9 5	110	22	2	9	22	15	18	12	147	89	120
11 1	108										
5 1	52	18	−4	−2	12	3	7		118	80	98
7 2	61	20	−5	−2	16	3	8		130	90	105
10 0	112	27	1	6	24	9	17	11	130	85	106
11 6	129	35	−3	5	26	9	18		121	83	100
5 5	49	18		−5	17	0	8		97	72	90
7 1	9										
9 5	116	32	0	[illegible]	27	16	19	14	110	69	94
5 1	58	18	−5	−3	13	[illegible]	5		109	76	94
10 [illegible]	117	28	−1	6	27	9	18		118	79	99
7 8	[illegible]5	19	−6	−5	16	4	9		114	80	97
8	100	28	0	8	25	7	15	10	98	71	87
8 0	99	30	1	7	24	7	17		104	74	88
10 8	12	26	0	4	25	9	14	10	120	81	94
12 3	121				29	16	21	10	116	77	100
8 8	106	28	1	8	23	11	16	10	132	72	98
11 2	123	28	0	5	23	11	16		136	77	104
5 3	69	21	4	8	20	9	14	10	101	69	85
7 [illegible]	82				30	17	23	13			

TABLE III The effect of increased ventilation on some circulatory functions Mean values±stand tilted position (60°)

	Ventilation, l BTPS/min		Oxygen uptake, ml STPD/min		Heart rate beats/min	
	0°	60°	0°	60°	0°	60°
Hypervent	25 0	27 4	317	306	87	101
	±1 6	±1 4	±12	±12	±2	±4
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	±0 4	±1 4	±7	±11	±3	±6
Difference	16 5	17 7	43	41	9	5
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No of cases	12	8	12	8	12	8
Probability	<0 001	<0 001	<0 01	<0 01	<0 01	>0 4

that vigorous hypocapneic hyperventilation increases cardiac output to a variable extent and it has been discussed but not settled whether this rise is proportionate to the increased work of breathing or larger (5, 12, 15, 17) The purpose of the present study is to analyse the hemodynamic effects of increased ventilation at a relative isocapneic state accomplished by adding a large dead-space with minimal resistance to the airways of the subject With the aim to settle whether the mechanical effects of increased ventilation on the circulation might be more important in erect than in supine position the subjects were studied in both body positions

Material

Twelve patients (8 males and 4 females) referred to the hospital because of suspicion of cardiac disease, were investigated with cardiac catheterization Some anthropometric data of the subjects are given in Table I The majority had low frequency, early systolic murmurs over the pulmonary area of grade 1—3, evaluated as being of physiological origin Case no 11 volunteered for the study As judged by clinical findings, electrocardiogram, phonocardiogram, chest X-ray, exercise tolerance and the results of cardiac catheterization the circulatory dynamics were normal in all cases In no case could any left to right shunt or pulmonary stenosis be detected Static and dynamic spirometry was within normal borders in all cases

Methods and procedure

The procedure for right heart catheterization has been the same as earlier reported from this laboratory (3) Cardiac output was determined according to the direct Fick principle with collection of expired air with the Douglas bag technic

Cardiac output, intracardiac and intravascular pressures were determined during normal and artificially increased ventilation at rest in supine position and with the subjects tilted 60° head up on a tilt table A marked increase in ven

ard error of the mean and statistical significance of differences Recumbent (0) and head up

A V O_2 difference, ml/l		Cardiac output l/min		Stroke volume, ml	
0	60	0	60	0	60
32 3	47 4	10 0	6 5	115	66
±1 5	±1 7	±0 5	±0 4	±6	±5
34 5	53 7	8 2	5 1	105	57
±2 0	±3 8	±0 5	±0 4	±5	±8
−2 2	−6 3	1 8	1 4	10	9
±1 8	±2 5	±0 3	±0 3	±5	±5
12	8	12	8	12	8
>0 2	<0 05	<0 001	<0 01	>0 05	>0 1

tilation was achieved by having the subjects breathe through a large bore hose thus increasing the respiratory dead space The dead space added was approximately 950 ml and consisted of a polyethylene hose with an inner diameter of 31 mm A flow of about 100 l min could be maintained through the hose with a pressure difference less than 1 cm H_2O With the purpose to avoid *influence of apprehension the subjects* were thoroughly informed about the procedures and also accustomed to the tilt table to breathe through the breathing valve and the dead space prior to the investigation Determinations of pH and pCO_2 of the arterial blood in 5 cases showed that the subjects adapted easily to the increase of the dead space through increases of tidal volume and often also of breathing rate so that they remained essentially isocapneic The subjects were *allowed a minimum of 5 minutes to* reach new circulatory and respiratory equilibrium after inducing changes in body position and ventilation Eight subjects were investigated at rest during normal and increased ventilation both in a supine and in a 60° tilted position Four subjects were only studied in the supine position

Results

Individual values are presented in Table II and some mean values and statistical significance of differences in Table III

In the *supine position* artificial increase of the dead space by 950 ml caused an average rise of the minute ventilation from 8 5 l BTPS min to 20.5 l BTPS min (Table III) but the individual variations were marked (Fig 1) The ventilatory increase was accompanied by significant increases of oxygen uptake (16 per cent) heart rate (11 per cent) and of cardiac output (22 per cent see Fig 2) The arteriovenous oxygen difference decreased insignificantly by an average of 6 % per cent The stroke volume increased on the average by 10 per cent but the change was not significant

TABLE III The effect of increased ventilation on some circulatory functions Mean values ± stand tilted position (60°)

	Ventilation l BTPS/min		Oxygen uptake, ml STPD/min		Heart rate beats/min	
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In most studies in man designed to elucidate the effect of respiration on the circulatory dynamics voluntary hyperventilation resulting in hypocapnia has been employed (8 9 11, 13 14 16 17) This technic is open to criticism because of the obvious difficulties to secure a relative steady state Furthermore in several studies less reliable indirect methods for determination of cardiac output have been used

In the present study increased ventilation was achieved by having the subjects breathe through a hose with minimal resistance which increased the anatomical dead space by 950 ml and cardiac output was determined during a relative steady state according to the direct Fick principle With this technic a significant rise in cardiac output occurred with increased ventilation due to an increase of heart rate and in most cases also of stroke volume Case no 5 showed the most marked increase of stroke volume (46 per cent) and of cardiac output (65 per cent) The increase in cardiac output with increased ventilation was more pronounced than could be explained by the larger work of breathing This is illustrated by Fig 3 which shows that

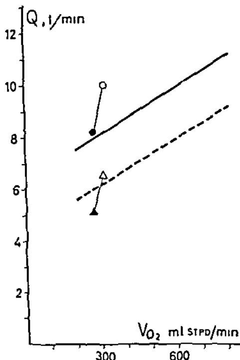


Fig 3 Cardiac output (Q) in relation to oxygen uptake (V_{O_2}) in recumbent position at rest during normal (●) and increased total ventilation (○) and in 60° head up tilted position during normal (▲) and increased ventilation (△) The regression lines for the increase in cardiac output in relation to the increase in oxygen uptake with leg exercise for normal subjects (3) are given for supine (unbroken line) and sitting body position (broken line)

with hyperventilation the cardiac output increases considerably more than with a leg work resulting in the same oxygen uptake Normally with exercise the arteriovenous oxygen difference increases and the increase is steeper with mild exercise than with severe exercise With increased ventilation on the other hand the arterio-venous oxygen difference remained unchanged or decreased This lack of widening of the arterio-venous oxygen difference during hyperventila

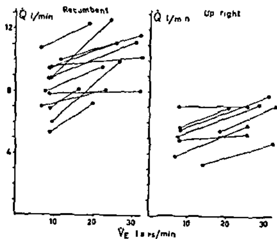


Fig 1 Effect of increased total ventilation (V_E) at rest on cardiac output (Q) in recumbent and 60° head up tilted position

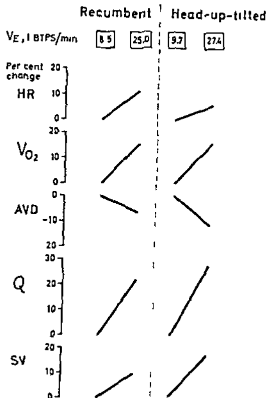


Fig 2 Effect of increased ventilation (V_E) at rest in recumbent and 60 head up tilted position on heart rate (HR) oxygen uptake (V_{O_2}) arterio venous oxygen difference (AVD) cardiac output (Q) and stroke volume (SV) The effect is expressed as average per cent change for the whole material

The systemic arterial mean pressure did not change significantly with increased ventilation Thus, the calculated systemic vascular resistance decreased The mean pulmonary artery pressure was also essentially unchanged The respiratory variations of the systemic and pulmonary arterial pressures and of right ventricular diastolic pressure, increased markedly with the increase in total ventilation indicating larger swings in intrathoracic pressure with increasing tidal volume

In the 60° *head-up tilted position* the total ventilation increased from 9 7 l BTPS min to 27 4 l BTPS/min when the dead space was added This resulted in approximately the same absolute increases in oxygen uptake and cardiac output which were statistically significant (Table III) There was a slight but not significant increase in heart rate with increased ventilation in the head up-tilted position The arterio venous oxygen difference decreased more than in supine position and the change was probably significant (Table III) The increases in cardiac output and stroke volume as well as the decrease in arterio-venous oxygen difference were relatively more important with increased ventilation in the head up tilted position than in the supine position (Fig 2)

Discussion

The mechanical effects of the so called abdominothoracic pump would favour acceleration of blood flow toward the right ventricle with each inspiration when intra thoracic pressure decreases

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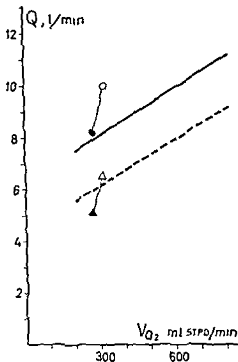


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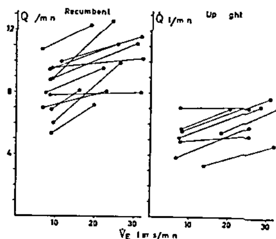


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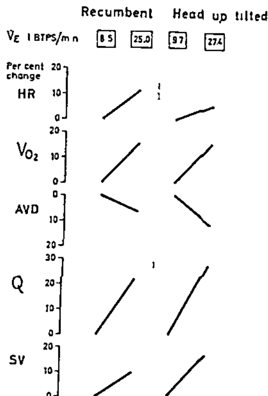


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tion was observed already by Grollman (9) who suggested that the blood flow change was not wholly the result of the metabolic effects of increased ventilation

The mechanisms for the rise in cardiac output with increased ventilation are not settled If the mechanical effect of the so-called abdomino-thoracic pump should be the most important factor, it might be expected that increased ventilation should give a more marked increase in cardiac output when this is low as in the upright position

When the subjects were tilted from horisontal to a 60° head up position the cardiac output decreased on the average by about 3 l/min The extent to which the cardiac output will decrease in the erect position depends largely on the degree of relaxation of the subject This may explain why there was a larger difference in cardiac output between the two body positions in this material than earlier found between supine and sitting position (3) The rise in cardiac output with increased ventilation was relatively more important in the up right than in the recumbent position, but of the same absolute order in both body positions

Summary

In twelve patients with normal circulation the hemodynamic effect of increased ventilation was studied in recumbent and 60° head-up tilted position Increased ventilation in a relative isocapneic state was accomplished by adding a dead space of about 950 ml to the airways of the subject Cardiac output was determined according to the direct Fick principle and intracardiac and intravascular pressures were measured

With this increase of the dead space the ventilation rose to an average of 25 l BTPS/min in recumbent position and was accompanied by significant increases of oxygen uptake (16 per cent), heart rate (11 per cent) and cardiac output (22 per cent) The arterio venous oxygen difference decreased and the stroke volume increased but the changes were not significant The cardiac output increased more than could be expected from the increase in respiratory work, as reflected in the oxygen uptake

In the up right position the increase in cardiac output was of the same absolute order as in recumbent position but was relatively more important and there was a probably significant decrease of arterio-venous oxygen difference

The results suggest that a significant portion of the increase in cardiac output during exercise could result from the increase in ventilation

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Methods

The patients were studied in the morning by heart catheterization with one catheter advanced from a cubital vein to the pulmonary artery and one catheter percutaneously introduced in a brachial artery. Pressures were recorded with ELEMA EMT 490 A transducers. Cardiac output was measured according to the direct Fick method. Oxygen saturation was measured spectrophotometrically (14). Blood volume was measured by means of I^{131} albumin with a Volumetron apparatus (6). The degree of red cell aggregation was estimated in vivo by microscopy of conjunctival vessels. The changes were classed in three grades. + denotes slow capillary flow and a few aggregates, + + denotes very slow flow in capillaries, several aggregates in capillaries as well as in the arterioles and venules, + + + denotes large aggreates in all vessels of the bulbar conjunctiva and cessation of flow in several places.[1] Lactic acid was determined in the first two cases by a calorimetric method (2), in the rest of the cases by an enzymatic method (16).

Immediately after those measurements LMD was infused, usually in an amount of 10—15 % of the initial blood volume and with an infusion rate of 15—20 ml per minute. After the infusion the measurements were repeated. In one patient (case no 2) who hypovolemic a larger amount of LMD was infused and cardiac outpuut was determined twice during the infusion.

The pulmonary resistance was calculated as the difference between the mean

[1] Conjunctival microscopy was performed by Dr Birgitta Zetterstrom.

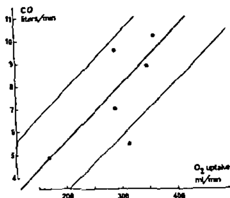


Fig. 1. Cardiac output and O_2 uptake at rest prior to LMD infusion in seven patients with marked red cell aggregation (filled circles). Regression line ± 2 SD for healthy young men in recumbent position.

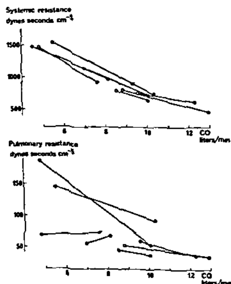


Fig. 2. Resistance — flow relationship in seven patients with marked red cell aggregation before (open circles) and after (closed circles) LMD infusion.

pulmonary artery and pulmonary capillary venous (PCV) pressures divided by the cardiac output, the systemic resistance

From the Department of Internal Medicine the Department of Clinical Physiology and the Department of Surgery, Karolinska Sjukhuset, Stockholm

Hemodynamic findings in patients with intravascular red cell aggregation

By

L Engstedt, U Freyschuss, L Kaijser, P Åsen

In recent years the flow properties of blood have awoked great interest (13) The reason is that in many pathological conditions an increased blood viscosity occurs This depends mainly on an increased red cell aggregation caused by plasma protein changes The condition is seen especially after trauma

While an increased blood viscosity is shown in vitro significantly to change the flow properties, less is known about the real importance of the change in clinical conditions (8 9, 19) In most patients with a red cell aggregation and an impaired tissue perfusion other factors may contribute to the perfusion disturbance for example hypovolemia

The aim of this study was to see if any deviations from normal function occurs in patients with a marked degree of red cell aggregation without other abnormalities which could influence hemodynamics and tissue metabolism Our second objective was to see if a reduction of the aggregation could normalize any pathological findings in a patient To reduce the aggregation we have thus infused low molecular weight dextran (LMD)[1] which substance has a well documented desaggregating effect, at least when measured in vitro Though not a main objective of the investigation we have observed some effects of dextran infusion which are not caused by its desaggregating effect

Material

Seven patients with an erythrocyte sedimentation rate (ESR) of more than 80 mm per hour were studied The red cell aggregation was caused in five cases by surgical repair of a fracture or non union of femur or tibia and in two cases (cases no 6 och 7) by paraproteinaemia The surgical patients were studied 3—5 days after the operation when the sedimentation rate had reached its maximum Except red cell aggregation the patients were from a circulatory and respiratory point of view healthy with one exception case no 7 had signs of coronary disease (typical angina pectoris and pathological ST depressions on the ecg during work)

[1] Rheomacrodex 10 % in dextrose Pharmacia

aggregation before and after aggregation reducing therapy (LMD infusion)

Pressure mm Hg								Pulse Rate c/sec	CO liters/min	SV ml	Res_s Dyn sec	Res_p cm *
RA	PA			PCV	A Brach							
	S	D	M		S	D	M					
4	21	9	14	9	125	72	91	95	8 58	90	808	46
8	24	12	19	13	122	69	90	104	10 07	97	656	39
5	25	14	20	10	138	90	112	100	5 48	55	1552	146
	31	17	21		139	95	112	112	9 35	84	912	
14	41	24	34	22	*142*	*87*	*110*	*110*	10 3	94	744	94
2	20	10	14	8	121	69	91	100	8 85	89	808	54
7	34	19	27	19	122	68	85	105	*12 96*	*123*	*480*	37
2	19	10	14	7	128	73	94	107	9 60	90	768	58
7	27	16	21	15	145	80	104	108	12.29	114	632	39
3	23	6	14	9	135	75	104	72	7 00	97	1152	57
8	30	13	21	14	145	77	107	79	8 14	103	976	69
3	18	7	12	8	110	66	87	78	4 85	59	1472	70
10	32	20	26	19	125	72	98	91	7 62	84	928	74
6	25	13	18	11	125	82	98	75	10 11	135	728	55
11	44	21	32	21	134	85	103	77	4 74	62	1552	186

normal Lactate concentrations were normal in central venous blood

Findings after infusion of LMD

After a moderately fast infusion of LMD the blood volume increased about 30 % more than the infused dextran volume. Signs of diminished red cell aggregation were seen the ESR was as a mean 21 mm less than before the infusion and there was in some cases a slight reduction in the degree of aggregation in the conjunctival vessels No significant change in lactate concentration, oxygen uptake or arterial oxygen saturation was found

The right atrial and PCV pressures rose considerably Cardiac output increased significantly in all cases except one. The increase was mainly caused by an increased stroke volume and to a lesser degree by an increased pulse rate The arterial pressure was unchanged In one patient (case no 7) there was a considerable reduction in cardiac output, in spite of right atrial and PCV pressure increase The hypovolemic patient could be given a large quantity of

TABLE I Descriptive data and hemodynamic findings in seven patients with marked red

Case No	Age years	Infus LMD ml	BV liters	Hct %	ESR mm/hr	Aggreg	Oxygen uptake ml STPD/min Pred	Obs	Lactate mEq/liter
1	♂	0		34	113	++	280	285	0 55
	34	500		29	95	+	280	292	0 90
2	♂	0	4 6	42	84	+++	292	313	0 53
	46	1200				++	292	316	
		1400	5 9	33	66	++	292	344	1 23
3	♂	0	5 4	42	83	++	267	348	0 39
	27	650	6 2	29	85	++	267	398	0 79
4	♂	0	4 9	36	104	++	241	289	0 35
	24	500	5 7	32	87	+	241	295	0 38
5	♂	0	5 5	33	90	+	259	290	0 68
	52	550	6 8	28	70	+	259	288	0 49
6	♀	0	4 1	34	100	++	172	171	
	55	600	5 3	28	68	+	172	182	
7	♂	0	7 8	32	112	++	297	361	1 2
	51	900	8 3	29	66	++	297	354	1 1

as the difference between the mean brachial artery and right atrial pressures divided by the cardiac output and expressed in C G S units

Results

Hemodynamic and metabolic findings at rest

The findings are summarized in Table I The oxygen uptake was 14 % larger than the values calculated according to Harris and Benedict and corrected for an elevated body temperature that was found in some of the patients (highest body temperature 38 7° C) One patient had a somewhat small cardiac output and even a somewhat low arterial oxygen saturation This patient had a small blood volume in relation to his body weight All the other patients had normal cardiac output in relation to the oxygen uptake (Fig 1) (15) All patients showed normal pressures in the right atrium and ventricle pulmonary artery, PCV position and brachial artery The pulmonary and systemic resistances were

fusion are described earlier (7, 10, 17). An interesting finding described but not commented by other authors is that the increased cardiac output was caused by an increase both in stroke volume and pulse rate. When the filling pressures for the heart are increased by head down tilting or by infusion of blood, the stroke volume is increased but the pulse rate is unchanged or decreased (12). The finding may indicate that dextran has a specific pharmacological effect beside the plasma volume expanding effect. The same finding is seen in normal subjects after dextran infusion (7, 10, 17).

The systemic resistance was found to vary inversely with the cardiac output as is to be expected in an actively pressure regulated system. The pulmonary resistance remained mainly unchanged with increasing cardiac output. This might indicate that the pulmonary vessels do not passively adapt to flow changes (18).

In one patient, contrary to the others the elevated right atrial and PCV pressures were accompanied by a decreased cardiac output. This patient was a coronary patient and the finding seems to be an example of the disadvantageous effect of rapid plasma volume expansion in a cardiac patient.

Summary

1. *In seven patients with marked intravascular red cell aggregation as judged from ESR and conjunctival microscopy no signs of an impaired tissue oxygenation were found.*

2. All patients showed normal pressures, flows and resistances in the circulatory system except one case who showed a somewhat small cardiac output. This was caused by hypovolemia.

3. *Infusion of* LMD decreased the in vitro signs of red cell aggregation and to a slight degree even the in vivo signs. Still the effect of the infusion on oxygen uptake and circulation were the same as described in normal subjects.

4. The elevated right atrial and PCV pressures were accompanied by an increased stroke volume and pulse rate, the later finding deviating from what is seen when elevated filling pressures are caused by tilting or blood infusion. This may suggest a specific pharmacological effect of dextran.

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LMD before extreme pressure changes occurred

The systemic resistance decreased, but the pulmonary resistance showed no significant change (Fig 2)

Discussion

The oxygen uptake, 14 % more than the predicted value, agrees well with earlier findings in patients during heart catheterization (4) Comparison with oxygen uptake in the same patients without red cell aggregation could not be made as some of the patients (cases no 6 and no 7) maintained their aggregation No significant change was seen when the degree of aggregation was diminished by LMD infusion Thus no diminished oxygen uptake caused by the red cell aggregation could be found If an impaired tissue perfusion existed, an elevated lactate concentration should have been found, or if in some tissue compartments the circulation was completely stopped, acid metabolites, for example lactate, should have been trapped and dislodged after desaggregation (1, 3) However, the lactate concentration was normal in the resting state and was not significantly influenced by LMD infusion Thus no signs of impaired tissue oxygenation were found Pressures in the circulatory system and calculated pulmonary and systemic resistances were normal in all cases Only one case (case no 2) showed a slight deviation from normal circulation This patient had a small cardiac output in relation to the oxygen uptake The patient had a very small blood volume in relation to his body weight The small cardiac output could have been caused by a high blood viscosity or the decreased blood volume If a high viscosity was the cause, it seems likely that a decreased oxygen uptake should have been found as well as an augmented arterial pressure caused by the augmented perfusion resistance Because of the small blood volume this patient was given more LMD than the others and cardiac output was measured twice during the infusion Infusion of 1200 ml increased the cardiac output to a normal value in relation to the oxygen uptake without influencing the pressures in the right ventricle or pulmonary artery It seems likely that this represents the effect of normalizing his blood volume Not until more LMD was infused did he show elevated right atrial and PCV pressures like the rest of the cases Thus the most probable cause of the lowered cardiac output was a decreased blood volume

After infusion of LMD there was a definite lowering of the sedimentation rate but a very slight decrease of red cell aggregation in the conjunctival vessels This is in accordance with the findings of other authors that there is often a good correlation between in vitro (ESR) and in vivo signs of red cell aggregation (11) but that deviations from this rule are seen (5) There was no significant change in lactate concentration and no significant increase in oxygen uptake This further supports the hypothesis that no or very slight circulatory and metabolic changes in the patients were caused by the red cell aggregation

The marked increase in right atrial and PCV pressures together with an increased cardiac output after dextran in

From the Department of Clinical Physiology Karolinska institutet Serafimerlasarettet Stockholm Sweden

A Simplified Procedure for the Calculation of Cardiac Output from Dye Dilution Curves[1]

By

Lennart Jorfeldt and John Wahren

Following the discovery of rapidly eliminated plasma bound dyes and the development of equipment for continuous recording of dye concentrations in blood, the indicator dilution method has become widely used for the determination of cardiac output The arterial time concentration curve recorded after an intravenous injection of indicator has been subjected to mathematical analysis and description by Meier and Zierler (6) The downslope of the curve is considered to be monoexponential until the start of recirculation (3) and the area under the curve for the first circulation of the indicator is calculated accordingly The conventional procedure for area determination involves replotting of the original curve in a semilogarithmic system the last part of the downslope being extrapolated in order to exclude recirculating indicator The coordinates for the extrapolated part of the primary circulation curve thus found are then transferred back to the original tracing and the area under the curve is determined with a planimeter This area in units of concentration × time is then used in the calculation of cardiac output

This procedure for measuring the area under the curve is rather laborious and time-consuming especially as a number of curves are usually recorded in the course of a single study Several attempts have been made to simplify this determination (9, 8 1) It is the aim of the present report to suggest a less complicated procedure for area determination based simply on certain linear dimensions of the original curve

Material and procedure

The study is based on calculations from 40 dye dilution curves Of these 30 were obtained from 6 healthy volunteers and 10 from 10 cardiac patients Five curves were recorded at varying levels of cardiac output from each of the six healthy subjects The volunteers were subjected to a thorough clinical investigation and

[1] A preliminary account of these results was given at the Xth meeting of the Scandinavian Society for Clinical Chemistry and Clinical Physiology 1965 (4)

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The integration interval is the same length (2 h) for each parabola segment. R is an estimation of the truncation error and has been omitted in these calculations.

Area B is inscribed into a trapezium of height $(X_{0.4}-X_{0.6})$ and having the sides $Y_{0.6}$ and $Y_{0.4}$. The subscripts indicate that the coordinates are those for the points on the curve corresponding to 0.6 and 0.4 of the peak concentration (Y_{peak}). The area of this trapezium (T) is

$$T=(X_{0.4}-X_{0.6})\ \frac{Y_{0.6}+Y_{0.4}}{2} \qquad (1)$$

Area B can be demonstrated to be a constant fraction of T. The downslope of the dye curve is assumed to be a monoexponential function

$$Y=k\ e^{-aX} \qquad (2)$$

Both $(X_{0.6}, Y_{0.6})$ and $(X_{0.4}, Y_{0.4})$ satisfy (2). When solved for X the equations become

$$X_{0.6}=\frac{1}{a}(\ln k-\ln Y_{0.6}),\ X_{0.4}=\frac{1}{a}(\ln k-\ln Y_{0.4})$$

Area B is

$$B=\int_{X_{0.6}}^{X_{0.4}} k\ e^{-aX}\,dX=\frac{1}{a}(ke^{-aX_{0.6}}-ke^{-aX_{0.4}})=\frac{1}{a}(Y_{0.6}-Y_{0.4}) \qquad (4)$$

After insertion of (3) into (1) the ratio of areas B and T becomes

$$\frac{B}{T}=\frac{2(Y_{0.6}-Y_{0.4})}{(Y_{0.6}+Y_{0.4})\ln\frac{Y_{0.6}}{Y_{0.4}}}=\frac{0.4}{\ln 1.5}\sim 0.986 \qquad (5)$$

$$B\sim 0.986\ T \qquad (6)$$

Area C is

$$C=\int_{X_{0.4}}^{\infty} ke^{-aX}\,dX=\frac{1}{a}ke^{-aX_{0.4}}=\frac{1}{a}\ Y_{0.4} \qquad (7)$$

It follows from (4) that area C is twice area B. Areas B + C may then be calculated as

$$B+C=3\ B=3\cdot 0.986\ \frac{Y_{0.6}+Y_{0.4}}{2}\ (X_{0.4}-X_{0.6})=1.48\ Y_{peak}\,(X_{0.4}-X_{0.6}) \qquad (8)$$

Procedure

To calculate the area under the dye dilution curve the abscissa of area A between the first appearance of dye (t_0, Fig. 1) and the peak dye concentration (t_4) was divided into four equal parts. The coordinates t_{0-4} were noted on the abscissa. t_4 was then measured and 0.6 (y_6) and 0.4 (y_7) of this value were

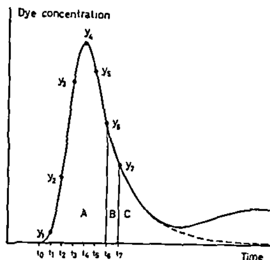


Fig 1 Model indicator dilution curve with the extrapolated part of the primary circulation of the dye indicated by the broken line Area A is the area under the curve between the first appearance of dye and until the descending limb of the curve has reached 0 6 of the peak dye concentration The area under the curve between 0 6 and 0 4 of the peak concentration is area B and the remainder is area C The peak of the dye curve is indicated on the abscissa (t_4) and the abscissa between the appearance of dye (t_0) and t_4 is divided into four equal parts defined by t_1 t_2 and t_3 0 6 and 0 4 of the peak concentration are marked on the abscissa (t_6 and t_7) and t_5 is placed half way between t_4 and t_6

considered free of cardiovascular disease The patients were referred to this laboratory for heart catheterization and all had various cardiac disorders The diagnoses were aortic stenosis, mitral and aortic stenosis, combined mitral in competence and stenosis (predominant stenosis), aortic aneurysm, status post myocardial infarction, Wolf Parkinson White syndrome, status post myocarditis, paroxysmal supraventricular tachycardia None of the patients had intracardiac shunts or was in congestive heart failure

Catheters were introduced percutaneously into a brachial artery and a medial cubital vein The venous catheter was advanced until the tip was calculated to be in the axillary vein or the superior vena cava Indocyanine green (5 mg) (Cardio green, Hynson, Westcott and Dunning, Baltimore, U S A) was injected through the venous catheter with a special syringe according to Sparling et al (7) and Grimby and Nilsson (2) The dye dilution curves were recorded with a densitometer (Waters X 300, Waters Corporation, U S A) and a potentiometric recorder (Speedoma , type G, Leeds and Northrup, U S A) The time for full scale deflection of the recorder was 0 3 sec Blood was drawn through the densitometer cuvette at a constant rate of not less than 14 ml min

Calculations

Theory

The area under the curve for the primary circulation of the indicator was divided into three parts, A B and C (Fig 1) A is the area under the curve between the first appearance of dye and until the dye concentration has declined to 0 6 of the peak B is the area under the descending limb of the curve between 0 6 and 0 4 of the peak concentration and C is the remainder of the area under the curve for the primary circulation of indicator

As illustrated in Figure 1 the dye-curve in *area A* may be approximated by three parabolas The areas between the abscissa and these parabolas can then be calculated from Simpson s formula

$$\int_{X_0}^{X_{2n}} f(X)\ dX = \frac{h}{3}(Y_0 + 4Y_1 + 2Y_2 + 4Y_3 + 2Y_4 + \quad 4Y_{2n-1} + Y_{2n}) + R$$

The integration interval is the same length (2 h) for each parabola segment. R is an estimation of the truncation error and has been omitted in these calculations.

Area B is inscribed into a trapezium of height ($X_{0.4} - X_{0.6}$) and having the sides $Y_{0.6}$ and $Y_{0.4}$. The subscripts indicate that the coordinates are those for the points on the curve corresponding to 0.6 and 0.4 of the peak concentration (Y_{peak}). The area of this trapezium (T) is

$$T = (X_{0.4} - X_{0.6})\,\frac{Y_{0.6} + Y_{0.4}}{2} \qquad (1)$$

Area B can be demonstrated to be a constant fraction of T. The downslope of the dye curve is assumed to be a monoexponential function

$$Y = k\,e^{-aX} \qquad (2)$$

Both ($X_{0.6}$, $Y_{0.6}$) and ($X_{0.4}$, $Y_{0.4}$) satisfy (2). When solved for X the equations become

$$X_{0.6} = \frac{1}{a}(\ln k - \ln Y_{0.6}) \quad X_{0.4} = \frac{1}{a}(\ln k - \ln Y_{0.4})$$

Area B is

$$B = \int_{X_{0.6}}^{X_{0.4}} k\,e^{-aX}\,dX = \frac{1}{a}(k e^{-aX_{0.6}} - k e^{-aX_{0.4}}) = \frac{1}{a}(Y_{0.6} - Y_{0.4}) \qquad (4)$$

After insertion of (3) into (1) the ratio of areas B and T becomes

$$\frac{B}{T} = \frac{2(Y_{0.6} - Y_{0.4})}{(Y_{0.6} + Y_{0.4})\ln\frac{Y_{0.6}}{Y_{0.4}}} = \frac{0.4}{\ln 1.5} \sim 0.986 \qquad (5)$$

$$B \sim 0.986\ T \qquad (6)$$

Area C is

$$C = \int_{X_{0.4}}^{\infty} k e^{-aX}\,dX = \frac{1}{a} k e^{-aX_{0.4}} = \frac{1}{a}\,Y_{0.4} \qquad (7)$$

It follows from (4) that area C is twice area B. Areas B + C may then be calculated as

$$B + C = 3\,B = 3 \cdot 0.986\,\frac{Y_{0.6} + Y_{0.4}}{2}\,(X_{0.4} - X_{0.6}) = 1.48\ Y_{peak}\,(X_{0.4} - X_{0.6}) \qquad (8)$$

Procedure

To calculate the area under the dye dilution curve the abscissa of area A between the first appearance of dye (t_0, Fig. 1) and the peak dye concentration (t_4) was divided into four equal parts. The coordinates t_{0-4} were noted on the abscissa. t_4 was then measured and 0.6 (y_6) and 0.4 (y_7) of this value were

calculated The corresponding x-coordinates (t_6 and t_7) were found on the abscissa and t_5 was found half-way between t_4 and t_6

The following measurements were made with a rule y_1–y_6, t_4—t_0, t_6—t_4, t_7—t_6 Area A was then calculated using Simpson s formula twice, first for the area with abscissa t_4—t_0 and then for the remainder of area A

$$A=(4y_1+2y_2+4y_3+y_4)\ (t_4-t_0)\frac{1}{12}+(y_4+4y_5+y_6)\ (t_6-t_4)\ \frac{1}{6}$$

Areas B and C were calculated from the formula

$$B+C=1\ 48\ \ y_4\,(t_7-t_6)$$

The values for A and B + C were then added to give the total area under the curve

Results

The dye dilution curves were calculated twice according to the conventional method, including semilogarithmic extrapolation and planimetry, and twice with the present technique The results are given separately for the groups of healthy subjects and patients in Table I

As will be seen, there is no loss of reproducibility with the present technique, in fact, slightly better values were found for the total area in both series of curves The curves from healthy individuals were divided into four groups with respect to the size of the area under the curve and the error of the method was calculated separately for each group No significant variations were found between the groups concerning the error of method with either procedure

With one exception, there were no significant differences between the subareas under the curves as measured with the two procedures The exception concerned area A in the curves from healthy individuals This area was found to be 1 7 per cent smaller ($p < 0\ 01$) with the present technique than when determined by planimetry As a result, the total area under the curve was 1 8 per cent smaller ($p < 0\ 01$) with the present technique

TABLE I The error of method in the determination of the total area under a dye dilution curve and its subdivisions for the present technique and the conventional procedure in a series of curves (n=30) obtained from healthy individuals and from patients (n=10) with various cardiac anomalies The error is expressed as the coefficient of variation for a singe determination

Group	Coefficient of variation per cent	
	Present technique	Conventional procedure
Healthy subjects		
Total area (A+B+C)	2 0	4 9
Area A	1 3	1 7
Area B	5 9	6 8
Area C	6 1	6 6
Patients		
Total area (A+B+C)	1 3	2 0
Area A	1 1	1 1
Area B	6 4	5 7
Area C	6 6	5 4

No such differences were found with the curves from patients with cardiac disorders

The descending limb of the dye dilution curve starts to deviate from a monoexponential slope when the recirculation of dye begins The level at which this deviation occurs was determined graphically in all curves and expressed as a percentage of the peak dye concentration For the curves recorded in healthy subjects the level was 20 2 ± 6 0 per cent of the peak (M ± SD) and for the curves from patients 23 8 ± 6 3 per cent The difference is not significant The highest value recorded in a curve from a healthy subject was 37 per cent

Discussion

The procedure suggested in this report for the calculation of the area under dye dilution curves proved easy to handle and was much less time consuming than the conventional method Moreover the necessary measurements can be obtained with a pencil and ruler and no special instrument is required for area determination

The technique is based to some extent on the principle used in currently available cardiac output computers It has a drawback in common with these computers on line determination of the area under the curve namely that the downslope is considered monoexponential down to a certain fraction of the peak concentration — in this study 0 4 — without this being checked in each curve This caused no error in the present study since all the curves in fact followed a monoexponential slope until well below 0 4 of the peak It was then assumed, in agreement with the traditional concept (5), that the remainder of the curve for the primary circulation followed the same course

The present technique was found to be equally applicable to curves obtained from patients with various cardiac anomalies as to those from healthy individuals It should be remembered, however, that the assumptions underlying the calculation of dye curves by the present technique as well as by the conventional procedure do not hold for curves recorded in patients presenting e g intra thoracic shunts or in congestive heart failure Care should always be exercised in the selection of curves, most of the abnormal ones can usually be detected by visual inspection

The present technique gave slightly lower areas (1 8 per cent) than the usual procedure for the curves recorded in healthy individuals This difference involved area A, which was computed according to Simpson s formula. The discrepancy is probably due to the approximation of the curve segments for area A to parabolas in Simpson s formula This error might possibly be eliminated by increasing the number of integration intervals for this area but since it is quite small and constant it may be more convenient to introduce a correction factor in the calculations.

Since the first presentation of the present method (4) a similar procedure has been suggested by Williams O Donovan and Wood (10) according to which the first part of the area also is calculated after approximation of the dye curve to three parabola segments Good

results were found for dye dilution curves with varying shape and area recorded in dogs

Summary

A simple procedure is suggested for the determination of the area under an indicator-dilution curve in an attempt to reduce the time needed for this calculation The procedure is based on certain linear dimensions of the original curve *The area under the curve from the first* appearance of dye until the dye concentration has decreased to 0 6 of the peak value is calculated according to Simpson's formula after approximation of the indicator curve to three parabola segments The remainder of the area can be shown to be equal to three times the area under the curve between 0 6 and 0 4 of the peak concentration This area, *in turn, may be determined as a constant* fraction (0 986) of the right angled trapezium into which the area becomes inscribed, when the points 0 6 and 0 4 on the curve are joined by a straight line

Forty dye dilution curves were calculated according to the conventional method including semilogarithmic extrapolation and planimetry and according to the present procedure Acceptable agreement between the methods and good reproducibility were found for curves recorded both from healthy individuals and from patients with cardiac disorders The present method was found to be much less time consuming

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From the Department of Clinical Physiology Lasarettet Lund Sweden

A Note on the Haemodynamic Effects of Nitroglycerine and Related Substances in Normal Subjects

By

I Nordenfelt and H Westling

It is generally agreed that nitroglycerine and related compounds can dilate all types of small vessels (see Charlier (3) for references) When given to man nitrates appear to cause mainly a relaxation of capacitance vessels, i e venules and veins (10 11, 12) The haemodynamic end result of this effect would be a reduction in venous return with a consequent drop in cardiac output and stroke volume

In a previous report from this laboratory (4) a reduction of the stroke volume during exercise was found when nitroglycerine was given to patients with coronary heart disease In addition its effects at rest were studied in patients with coronary heart disease and in normal subjects It was found that the drug diminished the resting stroke volume in patients whereas the normal subjects did not show any particular change after nitroglycerine The results indicated a difference under resting conditions between patients with coronary heart disease and normal subjects as regards the responsiveness to nitroglycerine However measurements during exercise were not available in normals

The present report contains supplementary observations of the effects of nitroglycerine during exercise in normal subjects In addition the effects on the heart rate and blood pressure of nitroglycerine and related substances were studied in normal subjects examined at rest in the recumbent and in the erect position The results support the belief that the effects of nitroglycerine in normal subjects are principially the same as in patients with coronary heart disease but that a quantitative difference in response may be explained by variations in venous tone under the conditions of the examination

Material and experimental procedure

The detailed haemodynamic effects of nitroglycerine were examined in five healthy subjects They were all males aged 22 33 37 37 and 51 years, and considered themselves healthy All had a normal physical working capacity with-

6 6633 5 ppl 4

out electrocardiographic changes in connection with an exercise test. In three of the subjects the effects of nitroglycerine were studied on three different work loads, 300, 600 and 900 kpm/min. Each subject was subjected to three separate exercise tests using one of the work loads. After a steady state had been reached, nitroglycerine was given and the response recorded. The subject then rested for about 45 minutes after which a second exercise test using a new load was performed. Nitroglycerine was again given when a steady state had been reached. After a further interval of 45 minutes a third test on the last work load was performed. The three subjects were thus subjected to three exercise tests, the order of which was varied so that a "latin square design" was achieved. In this way a comparison could be made between the effects of nitroglycerine during different work loads. In addition the possible effects of time on the response to exercise and nitroglycerine, and differences between subjects could be examined. The two remaining subjects were only studied during one work load, 300 kpm/min. For a comparison with the patients the five available responses on 300 kpm/min were pooled together and analysed.

Exercise was performed in the sitting position on a bicycle ergometer with electrical braking (5). The haemodynamic values during exercise before nitroglycerine were as a rule obtained during the fourth to sixth minutes of exercise, after which nitroglycerine was given. The response to nitroglycerine was evaluated about 4—6 minutes after giving the drug.

Nitroglycerine (Dumex) was given in a standard dose of 0,5 mg sublingually, regardless of the body weight. The other substances tested were given as described in the results.

The effect of posture on the responsiveness to nitroglycerine and related substances was studied in 13 normal males, aged 19—46 years. The subjects were examined lying on a couch or standing upright on the floor without support. Measurements of arterial blood pressure and heart rate were performed in the recumbent position and after 1, 2, 3, 4 and 5 minutes of standing. Thereafter the subject was asked to lie down again and rested for a variable time. Such successive orthostatic tests were performed two or three times before giving the drug in question and then repeatedly at varying intervals after the drug had been given. It was found that the changes in heart rate provided sufficient information for the purpose of the present study. The effects of different nitrates were studied separately on different days.

Methods

For detailed haemodynamic studies polyethene catheters were introduced into one brachial artery and one subclavian vein, using the percutaneous technique (1). Arterial pressures were recorded with the use of inductance manometers and registration on a direct writing electrocardiograph. The equipment of AB Elema, Sweden was used. Cardiac output was measured by the indicator dilution technique using bromsulphtalein as an indicator (9). Mean

TABLE I Haemodynamic observations during sitting exercise before (=B) and after (=A) 0 5 mg nitroglycerine sublinguany
Subjects 1—3 were examined three times at different work loads subjects 4 and 5 only once

Subject	Work kpm/min	Order of exercise		Heart rate	Cardiac output l/min	Stroke volume ml	Brachial artery pressure (mm Hg) Syst	Diast	Mean
1	300	III	B	112	8 8	79	94	53	69
			A	120	8 1	68	95	58	65
	600	II	B	144	13 3	92	123	61	74
			A	153	12 3	80	115	61	73
	900	I	B	156	14 9	96	159	69	98
			A	174	15 0	86	146	70	93
2	300	II	B	97	9 8	101	150	84	108
			A	117	9 0	77	132	84	108
	600	I	B	122	14 6	120	191	100	135
			A	150	13 6	91	177	102	119
	900	III	B	150	18 3	122	194	94	129
			A	174	15 6	90	185	95	123
3	300	I	B	84	12 5	149	148	95	120
			A	94	11 1	118	132	89	106
	600	III	B	114	14 0	123	128	78	102
			A	122	12 1	99	133	87	103
	900	II	B	130	20 2	155	164	88	120
			A	150	16 4	109	158	92	112
4	300	—	B	120	11 2	93	150	94	114
			A	142	9 6	68	131	90	100
5	300	—	B	81	10 5	130	134	75	97
			A	102	8 0	78	115	71	86

arterial pressures were obtained by electrical integration

For measurements of the effect of posture on the responsiveness to nitrates indirect measurements of arterial blood pressure were made by the auscultatory method The electrocardiogram was recorded to obtain the heart rate

Results and comments

1 Effects of nitroglycerine during exercise

The primary observations are given in Table I The average changes after nitroglycerine in 5 subjects studied at 300 kpm/min are given in Table II It may be seen that the dominant changes are tachycardia reduced stroke volume and a fall in systolic blood pressure The arterial mean pressure was not much reduced this means that the calculated total peripheral vascular resistance was slightly increased since the cardiac output fell slightly more than the mean pressure

The results obtained in subjects 1—3 showed in addition that the effects of nitroglycerine on heart rate cardiac out-

TABLE II A comparison of the haemodynamic effects of nitroglycerine during exercise in normal subjects and patients with coronary heart disease
All observations were made during exercise at a load of 300 kpm/min The detailed values for the patients were reported by Christensson et al (4)

	Initial value (mean values)		Change after nitroglycerine (mean ± S E)	
	Normals	Coronary heart disease	Normals	Coronary heart disease
	5 subjects	6 patients	5 observations	6 observations
Heart rate (beats/min)	99	99	+ 16 ± 3 0**	+ 25 ± 4 2**
Cardiac output (l/min)	10 0	8 5	— 1 4 ± 0 32*	— 1 4 ± 0 42*
Stroke volume (ml)	110	88	— 29 ± 6 7*	— 30 ± 3 5***
Arterial blood pressure (mm Hg)				
systolic	135	155	— 14 ± 3 8*	— 26 ± 5 8**
diastolic	80	81	— 1 8 ± 2 0	— 0 3 ± 2 4
mean	102	109	— 8 6 ± 3 1*	— 9 0 ± 1 1***

* ** and *** means a statistically significant change with p value less than 0 05 0 01 and 0 001, respectively

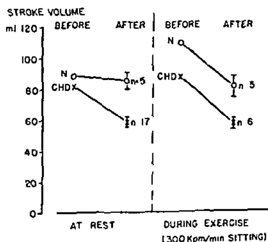


Fig 1 Comparison of the effects of nitroglycerine (0,5 mg sublingually) on the stroke volume at rest and during exercise in patients with coronary heart disease (CHD), × and normals (N) ○ The values for the normals (at rest) and the patients (at rest and during exercise) are taken from Christensson et al The bars indicate ± S E of mean difference n = number of observations

put and stroke volume tended to be larger at the highest work load Thus the average changes after nitroglycerine were an increase in heart rate of 13 beats/min and a decrease in cardiac output of 1,0 l/min at 300 kpm min, 15 beats min and 1,3 l/min at 600 kpm/min, and 21 beats/min and 2 2 l min at 900 kpm/min

From Table II it can also be seen that the response to nitroglycerine was largely the same as in the patients with coronary heart disease previously studied (4) These had lower initial values for cardiac output and stroke volume This difference is probably a sequel to their disease, but the fact that they were older than the normal subjects must also be borne in mind

Figure 1 shows a comparison of the effects of nitroglycerine on stroke volume

at rest and during exercise in normal subjects and in patients with coronary heart disease (4) The divergent response of the resting normal subjects is clearly seen The initial stroke volumes were approximately equal in the two groups, but nitroglycerine caused a fall only in the patients with coronary heart disease During exercise the stroke volume is higher in the normal subjects, now nitroglycerine causes a pronounced fall, about the same as in the patients previously investigated (4)

Corresponding differences in response were seen also in the other circulatory parameters studied

It may thus be concluded that normal subjects respond to nitroglycerine in principally the same way as coronary patients but that the effect is negligible at rest in the recumbent position The most natural explanation for this is that the supposed primary target mechanism of nitroglycerine, the venous tone, is not operating at rest in recumbency Exercise, especially in the sitting position, will increase venous tone also in normal subjects, (2) In the patients venous tone is likely to have been increased already at rest (6, 7)

It is well known that standing induces an increase in venous tone Correspondingly, it has been shown (10) that the circulatory effects of nitrates are much larger in the vertical position In the following section this fact was used in order to compare various 'nitrates'

2 Effect of nitroglycerine and related substances on the heart rate in the standing position

In normal subjects an ordinary dose of nitroglycerine hardly affects the heart rate and blood pressure in recumbency If the person stands up, however, tachycardia and a reduction in pulse ampli

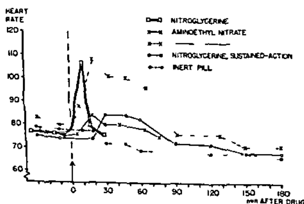


Fig 2 Comparison of the effects of various "nitrates" on the heart rate in the standing position. Each point represents the heart rate at an "orthostatic test" i e mean value during the 2nd—5th minutes of standing The drugs were given at "O" minutes Five experiments on different days in one subject

Nitroglycerine was given 0 5 mg sublingually aminoethyl nitrate 4 mg (×——×) and 8 mg (× – – – ×) sublingually Nitroglycerine "sustained action" 5,2 mg was swallowed and inert pill was given sublingually

tude are immediately apparent By means of such repeated "orthostatic tests" the effects of nitroglycerine can be followed conveniently and accurately We have used this technique to compare various "long-acting" nitrate preparations, which are reported to be effective against angina pectoris

Figure 2 shows the response of one subject to various preparations There was during the experimental period (about 4 hours) a successive decline in the heart rate during standing, as evident from the curve labelled "inert pill" The pronounced but short lasting effect of 0,5 mg nitroglycerine sublingually is in sharp contrast to the weak, more prolonged effect of a "sustained-action" preparation of the drug (Nitrong, Ethicals) in spite of the fact that the latter was given in twice (5,2 mg!) the commonly recommended dosage In these experiments only sublingually given drugs had an effect comparable to that of nitroglycerine, but more long lasting Figure 2 demonstrates such an effect of aminoethyl nitrate (Nilatil, Pharmacia) in a high dose (8 mg) The usually recommended dose (4 mg) was hardly effective This tallies well with the clinical observations of Soderstrom (8) who recommended a dose of 6—8 mg

Isosorbide dinitrate (Sorbangil, Kabi), pentaerythritol tetranitrate (Nitropent, Astra) and triethanolamine trinitrate biphosphate (Nitroduran, Lovens) were other drugs studied in the 13 normal subjects Isosorbide dinitrate (5—10 mg) administered sublingually had a well established effect Pentaerythritol tetranitrate, however, was ineffective in a dose of 30 mg (swallowed) and had a very weak effect even in twice that dose Another drug to be swallowed, triethanolamine trinitrate biphosphate, likewise had a very poor "orthostatic" effect in the recommended doses of 10 and 20 mg

Despite convincing pharmacological documentation of their efficacy and long duration it has been difficult to obtain clear-cut clinical results with several preparations of "long acting nitrates" given by the mouth and swallowed It seems fully possible that this is due to the simple fact that they are not absorbed in effective doses or that compensatory mechanisms counteract long lasting actions, cf (12) The present method of testing effects and duration of nitrate preparations in man would appear to be useful for the evaluation of new such drugs, as evidence for discarding some preparations, and for checking that the individual patient is given an effective dose The orthostatic test can be made in any physician s office

The validity of this type of evaluation rests entirely on the assumption that the effect of nitrates on the capacitance vessels is important for their therapeutic action

Summary

In normal subjects nitroglycerine sublingually during exercise reduces stroke volume and cardiac output and increases heart rate, just as in patients with coronary heart disease

It is suggested that these haemodynamic effects of nitroglycerine are more apparent under conditions with an increased venous tone

Repeated orthostatic tests might be used as a convenient method for quantitative evaluation of nitrate preparations

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Medizinische Universitatsklinik, Rontgen Radium Abteilung, Freiburg im Breisgau, Deutschland

Volumen und Leistung des gesunden und kranken Herzens

Von

H Reindell, K Musshoff, K Konig, W Gebhardt,
H Roskamm und J Keul

Unsere ersten Beobachtungen uber Beziehungen zwischen Herzgrosse und Leistung haben wir 1936 an Hochleistungssportlern gewonnen Es wurde festgestellt, dass besonders leistungsfahige Dauersportler uber vergrosserte Herzen verfugen Diese Herzvergrosserung bei Sportlern war erstmals von Henschen (1899) als Sportherz bezeichnet worden Seit dieser ersten Feststellung war die Auffassung uber den der Herzvergrosserung zugrunde liegenden Vorgang nicht einheitlich Die Großenzunahme wurde sowohl als Folge einer Muskelschadigung bzw einer latenten Insuffiziens (4, 10, 21, 22, 23, 36, 45, 46 47 u a), einer Inanspruchnahme von Reservekraften (35) als auch als physiologischer Anpassungsvorgang an die vermehrte korperliche Belastung gedeutet (6, 8 17, 31, 32, 37, 50, 58, 65, 68, 74 u a)

Die Auffassung, dass die Herzvergrosserung Folge einer Herzschwache sei, geschah durch Übertragung der klassichen Herzgesetze von Frank (9), Straub (67) und Starling (66), die damals alleinige Grundlage fur die Beurteilung des Herzens hinsichtlich Leistungsfahigkeit, Suffizienzgrad und Inanspruchnahme der Reservekrafte waren

Nachdem es seinerzeit nicht moglich war, die am Tierherzen gewonnenen klassischen Herzgesetze an Menschen zu uberprufen und gegebenenfalls zu revidieren, fehlten auch damals die physiologischen Voraussetzungen, in der Vergrosserung des Sportherzens gegebenenfalls auch einen Anpassungsvorgang zu sehen

Durch die rontgenologische Bestimmung des Herzvolumens (20, 59), durch Leistungsprufungen des Herzens, insbesondere durch blutige und unblutige Schlagvolumenbestimmungen, durch Messung der maximalen Sauerstoffaufnahmefahigkeit und durch intrakardiale Druckmessungen in Ruhe und unter Belastungsbedingungen (2) wurde es dann moglich, auch am menschlichen Herzen in Situ, ahnlich wie im Tierexperiment, die Beziehungen zwischen Grosse und Leistungsfahigkeit des suffizienten und insuffizienten Herzens zu uberprufen

Ein entscheidender Fortschritt in der Beurteilung des Funktionszustandes des Herzens war die Inbeziehungsetzung der röntgenologisch gemessenen Herzgroße mit der Gesamtleistungsfahigkeit des Organismus durch die Sjostrand sche Schule (63, 64) ein Untersuchungsverfahren, das wir mit einigen *Abanderungen uber*nommen haben

Die wesentlichen Grundsatze der eigenen Methode sind

1 Die Volumenbestimmung des Herzens in horizontaler Bauchlage wahrend Korperruhe nach einer modifizierten Rohrer Kahlstorf'schen Formel (40)

2 Die stufenweise Belastung am Fahradergometer im Liegen uber mehrere Zwischenstufen von je 6 Minuten bis zur maximalen Stufe ins relative steady state bzw in Ergostase (38)

3 Die Verwendung des O_2-Pulses auf dieser Belastungsstufe (maximaler O_2 Puls) als Maß der Leistungsfahigkeit

Die Beurteilung der kardio-zirkulatorischen Leistungsreserven erfolgte in Form *einer korrelativen Betrachtung von Herz*große und maximalen Sauerstoffpuls

In dieser auf den Arbeiten von Sjostrand und Mitarbeiter basierenden korrelativen Beurteilung liegt der grundsatzliche und entscheidende Unterschied gegenuber allen anderen in Deutschland entwickelten ergometrischen Methoden die nur die gemessene Leistungsfahigkeit als absoluten Wert fur die Beurteilung der Herzfunktion heranziehen Weitere von uns gemessene Werte wie Blutdruck Atemminutenvolumen Atemaquivalent und Ekg blieben fur die heutige Fragestellung unberucksichtigt Dagegen wer*den in der vorliegenden Arbeit* die Ergebnisse einiger Untersuchungsreihen *uber intrakardiale* Druckmessungen und Schlagvolumenbestimmungen zur Deutung der Befunde herangezogen

Unser heutiges zusammenfassendes Referat behandelt folgende Themen

1 Grösse und Leistungsfahigkeit des gesunden leistungsschwachen und lei*stungsstarken* Herzens

2 Grosse und Leistungsfahigkeit des suffizienten druck und volumenuberlasteten Herzens

3 Grosse und Leistungsfahigkeit des insuffizienten Herzens

Grosse und Leistungsfahigkeit des gesunden leistungsschwachen und leistungsstarken Herzens

1 *Ergebnisse* Als Maß der Leistungsfahigkeit des Herzens gelten das maximale Schlag und Minutenvolumen des Herzens das maximale Sauerstoffaufnahmevermogen und der maximale *Sauerstoffpuls*

Zwischen der Grosse des Herzens und der Grosse des maximalen Schlagvolumens besteht uber alle Grossenbereiche von kleinen untrainierten bis zu grossen trainierten Herzen eine enge lineare Beziehung Dabei findet sich eine gute Ubereinstimmung unserer Werte mit denen von Holmgren, Jonsson und Sjostrand (19) wie sowohl aus dem Vergleich der Einzelwerte (Abb 1) als auch der *Regressionslinien (Abb 2) hervor*geht Die geringen Unterschiede im Verlauf der Regressionslinien in Abb 2 erklaren sich z.T dadurch dass Holmgren et al die Mittelwerte der wahrend Belastung erreichten Schlagvolumina ver-

Medizinische Universitätsklinik, Röntgen Radium Abteilung, Freiburg im Breisgau, Deutschland

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rund 400 männlichen und 300 weiblichen Normalpersonen im Alter von 10 bis 75 Jahren sowie an 90 Sportlern überprüft (28, 44, 61) Die Untersuchungsergebnisse sind in Abb 4 dargestellt Der maximale Sauerstoffpuls erhöht sich direkt proportional zur Grösse des Herzens Kleine Herzen verfügen über eine geringe Leistungsbreite, grosse gesunde Herzen dagegen über eine erweiterte Leistungsbreite Innerhalb der einzelnen Lebensabschnitte bestehen signifikant bis hochsignifikant gesicherte lineare Korrelationen zwischen den Grossen Herzvolumen und Leistung (maximale O_2-Aufnahme pro Puls Von entscheidender Bedeutung ist jedoch dass die Beziehung zwischen Herzgrösse und Leistungsfähigkeit *in gesetzmässiger Weise vom Alter* verändert wird Diese Aussagen werden durch das Verhalten des sog „Herzvolumenleistungsquotienten verdeutlicht, der das Verhältnis von Herzvolumen zu max. Sauerstoffpuls in Abhängigkeit von Alter und Geschlecht wiedergibt (Tab 1)

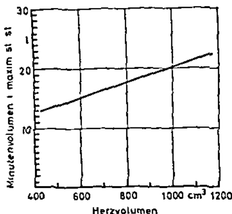


Abb 3 Die Beziehungen zwischen Herzvolumen (Im Liegen während Körperruhe bestimmt) und Minutenvolumen bei maximaler Belastung im steady state
$n = 26$ (9 Sportler 13 Männer und 4 Frauen)
Regressionsgleichung $y = 7.48 + 0.0129\,x$.
Korrelationskoeffizient $r = 0.529 \pm 0.141$
$(0.01 > P > 0.001)$
Aus Musshoff (39 b)

2 Zusammenfassung der Ergebnisse Die Ergebnisse einer korrelativen Betrachtung der Herzgrösse einerseits und der Leistungsmasse Schlagvolumen Minutenvolumen, Sauerstoffaufnahme und Sauerstoffpuls andererseits beweisen, dass

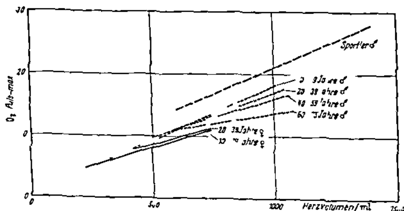


Abb 4 Regressionsgeraden der Korrelation zwischen Herzvolumen und maximalem Sauerstoffpuls bei den verschiedenen Altersgruppen männlicher und weiblicher Normalpersonen sowie bei Hochleistungssportlern Aus Reindell u Mitarb (49)

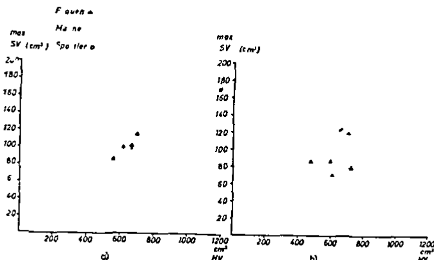


Abb 1 Die Abhangigkeit des maximalen Schlagvolumens wahrend Ergometerbelastung von der Herzgroße Von Holmgren u Mitarb (19), Bevegard u Mitarb 1 a (a), Reindell u Mitarb (56) (b) wurde einheitlich mit zunehmender Herzgroße eine Vergroßerung des maximal moglichen Schlagvolumen festgestellt Sportler mit sehr großem Herzvolumen zeigten ebenfalls die großten Schlagvolumina wahrend Belastung Aus Roskamm u Mitarb (60)

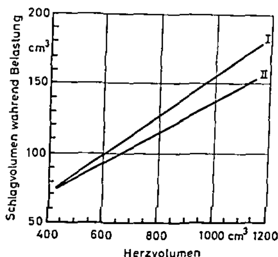


Abb 2 Die Beziehungen zwischen Herzvolumen (im Liegen wahrend Korperruhe bestimmt) und Schlagvolumen wahrend Belastung

I Musshoff, Reindell u Klepzig (41 42) Unter Verwendung der maximalen im allgemeinen Wahrend submaximaler Belastung erreichten Schlagvolumina
$n = 26$ (9 Sportler 13 Manner und 4 Frauen)
Regressionsgleichung $\gamma = 14{,}7 + 0{,}143\,x$
Korrelationskoeffizient $r = 0\,837 \pm 0\,059$
$P < 0\,001$)

II Holmgren Jonsson u Sjostrand (19) Unter Verwendung der Mittelwerte dar wahrend Belastung erhaltener Schlagvolumina
$n = 18$ (14 Manner und 4 Frauen)
Regressionsgleichung $\gamma = 27\,0 + 0\,11\,x$
Korrelationskoffizent $r = 0\,86$

Aus Musshoff (39 b)

wendet, wahrend wir den jeweiligen Maximalwert *einsetzen Dieselben* Beziehungen, wie zwischen Herzvolumen und maximalen Schlagvolumen, gelten in ahnlicher Weise auch fur die Korrelation Herzvolumen — maximalem Minutenvolumen (Abb 3)

Diese Untersuchungen beweisen, dass grosses Herzen unter Belastungsbedingungen grosse Schlag- und Minutenvolumen, kleine Herzen jedoch — entsprechend ihrer kleineren Grosse — kleinere Schlag- und Minutenvolumina auswerfen konnen Das grosse und das durch Sport und Training vergrosserte Herz ist — proportional seiner Grösse — ein leistungsstarkes, das kleine ein funktionell schwaches Herz

Die Gultigkeit dieser engen Beziehungen zwischen Grosse und Leistungsfahigkeit des Herzens wurde durch systematische grosse Reihenuntersuchungen mittels Herzvolumenbestimmung und spiroergometrischer Belastungsprufung an

Tabelle 11 *Arbeitsweise des gesunden menschlichen Herzens*

	Klassische Herzgesetze	Neue *Herzgesetze* *
in Ruhe	Kein oder nur geringes Restblut	*Restblut vorhanden* Restblut/Schlagvolumen ≈ 1:1 Kleines Herz ≈ 0,5:1 Sportherz ≈ 2:1
bei Belastung (Physiologischer Anpassungsvorgang)	Herzgrösse Zunahme (tonogene Dilatation)	Herzgrösse gleichbleibend oder Abnahme (systolisch und diastolisch)
Füllungsdruck	Anstieg	*kein Anstieg*

(gemeinsam mit Kleppzig und Steim)

im Gegensatz zu der Auffassung über die Arbeitsweise, Leistungsbreite und die Reservekraft des menschlichen Herzens, wie sie sich in Übertragung der klassischen Herzgesetze auf das menschliche Herz ableitet. In der folgenden Tabelle (Tab. 11) sind die Vorstellungen über die Arbeitsweise nach den klassischen Herzgesetzen und nach den neuerdings am menschlichen Herzen erhobenen Befunden gegenübergestellt. Im Vergleich zu den klassischen Befunden ergeben sich im einzelnen folgende Gegensätzlichkeiten:

1. Das gesunde menschliche Herz verfügt über eine erhebliche Restblutmenge, die umso grösser ist, je leistungsstärker das Herz ist (34, 48). Bei Herzen durchschnittlicher Größe und Leistungsbreite ist das Verhältnis von Restblut und Schlagvolumen 1:1. Bei kleinen leistungsschwachen Herzen ist das Restblut kleiner, beim großen leistungsstarken Sportherzen größer als das Schlagvolumen.

2. Der rechts- und linksseitige enddiastolische Füllungsdruck liegt bei allen gesunden Herzen unabhängig von der Herzgröße und der Größe der Restblutmenge innerhalb eines definierten Normbereiches. Eine Abhängigkeit zwischen *Grösse der Restblutmenge* und der Höhe des Füllungsdruckes besteht nicht. Abb. 5.

3. Körperliche Mehrarbeit bewältigt das menschliche Herz ohne Steigerung des diastolischen Füllungsdruckes (5, 18, 33, 51). Auch bei den vergrösserten Sportherzen ist während Belastung der diastolische Füllungsdruck nicht erhöht (52), *häufig sogar erniedrigt* (1 a).

4. Röntgenologisch zeigen die Herzen von Normalpersonen und Trainierten während geringer Belastung eine annähernd gleiche und bei stärkerer Belastung eine Verkleinerung der systolischen und diastolischen Endstellung (43, 54, 57, 62).

Der wesentliche Unterschied der Arbeitsbedingungen des *isolierten Herzens* und des menschlichen Herzens *in situ* besteht darin, dass der maximale Hubraum des menschlichen Herzens schon unter Ruhearbeitsbedingungen vorhan

Tabelle I *Der Quotient Herzvolumen/maximaler Sauerstoffpuls in Ergostase* bei männlichen und weiblichen Normalpersonen in den *verschiedenen Alters*bereichen und bei männlichen und weiblichen Sportlern

Alter in Jahren		10-11	12-13	14-15	16-17	18-19	20 29	30-39	40-49	50-59	60-75	*Sportler*
$\bar{x}$	m	57,6	55,6	56 7	53 1	49,1	55,2	56,5	57,9	59,7	67,6	46 6
	w	62,2	66,1	59,1	60,3	62,1	59 2	62,4	—	—	—	*57,8*
s	m	8,52	6,70	8 88	4,98	6,20	9 79	8 04	6,52	7,94	11,59	4 6
	w	5,71	8,03	7 55	8 33	11,80	8,70	7,82	—	—	—	8,3
$e\bar{x}$	m	1,39	1 06	1,42	0,74	0,89	1,46	1,29	1,36	1,53	1,78	0,48
	w	0 81	1 13	1 04	1,23	1 67	1,33	1 10	—	—	—	1,43
n	m	38	40	40	45	51	45	39	23	27	42	89
	w	50	50	50	46	50	43	50	—	—	—	34
Sign		xx	xxx	ø	xxx	xxx	x	xxx	—	—	—	xxx

(Mittelwert = $\bar{x}$, mittlere quadratische Abweichung = s, mittlerer Fehler des Mittelwertes = $e\bar{x}$, Anzahl = n Signifikanz = Sign)

die Leistung des gesunden Herzens mit zunehmender Grösse linear ansteigt. Das bedeutet, dass der Quotient Herzvolumen / max Sauerstoffpuls bei gesunden Herzen unterschiedlicher Grösse und Leistung mit folgenden Abweichungen grundsätzlich gleich ist

a) Bei Frauen ist die Leistung im Vergleich zur Herzgrösse etwas kleiner als bei Männern

b) Mit zunehmendem Alter nimmt die Leistungsfähigkeit des Herzen im Vergleich zur Herzgrösse vom 10 bis 20 Lebensjahr zu und jenseits des 20 Lebensjahres mit zunehmendem Alter zunächst gering, jenseits der 6 Lebensdekade jedoch erheblich wieder ab

Die engen Beziehungen, die zwischen Herzgrösse und körperlicher Leistung bestehen, bilden die Grundlage für eine klinisch brauchbare Funktionsdiagnostik des Herzens Erfährt bei einem Patienten die Korrelation von Herzvolumen und maximalem O_2-Puls eine derartige Abweichung, dass der der Altersgruppe zugehörige Normbereich überschritten wird, so besteht eine Belastungsinsuffizienz des Herzens, sofern extrakardiale Ursachen wie Regulationsstörungen, Anämien oder pulmonale Störungen ausgeschlossen sind Auf diese Weise ist es möglich, eine mit Glykosiden behandlungsbedürftige Herzinsuffizienz im Frühstadium zu erfassen, noch bevor Stauungssymptome oder Beschwerden auf ein Herzversagen hinweisen (27 30) Unter Glykosidbehandlung werden diese Herzen meßbar kleiner und leistungsstärker

3 *Deutung der Ergebnisse über Arbeitsweise und Leistungsbreite des Herzens* Die Feststellung, dass des grosse Herz ein leistungsstarkes, das kleine hingegen ein leistungsschwaches ist steht

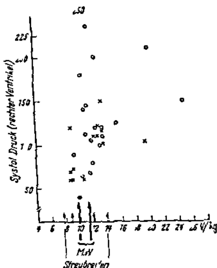


Abb 6 Pulmonalstenosen Systolischer rechter Ventrikeldruck Relative Herzgroße (V/kg) Manner ○ Frauen × Aus Reindell u Mitarb (49)

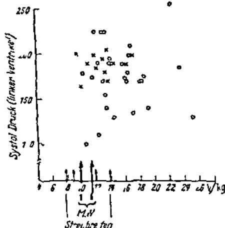


Abb 7 Aortenstenosen Systolischer linker Ventrikeldruck (Direktpunktion) Relative Herzgroße (V/kg) Männer ○ Frauen × Aus Reindell u Mitarb (49)

vermehrt druck und volumenbelasteten Herzens

Zur Beurteilung dieser Gegebenheiten haben wir die Beziehungen zwischen intrakardialen und intrapulmonalen Druckwerten, absoluter und relativer Herzgrosse sowie Leistungsfahigkeit bei rechts sowie linksseitig vermehrt druck und volumenbelasteten Herzen untersucht Als Beispiel der vermehrten Druckbelastung fuhren wir die Befunde der Aorten und Pulmonalstenose als Beispiel der vermehrten Volumenbelastung den Ductus Botalli die Septumdefekte und die Aorteninsuffizienz an.

I *Das druckuberlastete Herz*

1 *Ergebnisse* In Abb 6 finden sich die Werte fur den systolischen Ventrikeldruck und die relative Herzgrosse von 45 Pulmonalstenosepatienten Die Abbildung zeigt dass der rechte Ventrikel systolisch Drucke bis uber 200 mm Hg uberwinden kann ohne dass das Herz vergrossert ist Von den insgesamt 45 Herzen ist nur ein geringer Teil vergrossert und liegt ausserhalb des oberen Normbereiches Etwa 25 % der Herzen liegt sogar im unteren Normbereich

Abb 7 *enthalt die Druck Volumen*beziehungen bei 41 Patienten mit Aortenstenose (der Druck im linken Ventrikel wurde durch Direktpunktion (Doz Dr Steim) gewonnen) Auch hier ist keine Beziehung zwischen Herzgrosse und systolischer Druckbelastung festzustellen

Untersucht man die Grossenverhaltnisse des relativ*en Herzvolumens (Herz*volumen pro Korperoberflache), wie dies in Abb 8 fur verschiedene Formen druckbelasteter Vitien geschehen ist, so ergeben sich bei insgesamt 46 Patienten in 31 Fallen eine noch normale und nur bei 15 Fallen eine pathologische Herzgrosse

Abb 9 enthält die Beziehungen

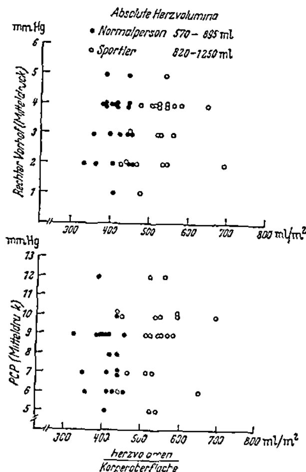


Abb 5 Druck Volumbeziehungen bei 20 Normalpersonen und 20 Sportlern Aus Reindell u Mitarb (40)

den ist und nicht erst durch vermehrte diastolische Dehnung mittels eines erhohten Fullungsdruckes geschaffen werden muss Neuere tierexperimentelle Befunde widerlegen die „klassischen" Herzgesetze und stehen in voller Über einstimmung mit unserer klinischen Konzeption (11, 12, 70, 73) Die Grosse des Hubraumes in Ruhe ist mitbestimmend fur die Leistungsfahigkeit des Herzens

Die Grossenzunahme des trainierten Herzens, die mit einer Vermehrung des Ruhe-Restblutes einhergeht, wurde von uns anfanglich als regulative Dilatation (50) und spater, da sie nicht nur mit einer Dilatation, sondern auch mit einer Muskelhypertrophie verbunden ist, als regulative Herzvergrosserung (55) bezeichnet Die Große des enddiastolischen bzw endsystolischen Volumens bestimmt somit unabhangig vom venosen Fullungsdruck die , potentielle Reserve kraft des Herzens

Grosse und leistungsfahigkeit des suffizienten druck- und volumenuberlasteten Herzens

Die im vorausgegangenen Abschnitt ge machte Feststellung, dass die Beziehun gen zwischen Herzgrosse und Leistung beim gesunden menschlichen Herzen nicht in Übereinstimmung mit den alten am isolierten Herzen gewonnenen Gesetz massigkeiten steht, gilt grundsatzlich auch fur die Arbeitsweise des suffizienten

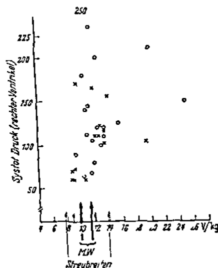


Abb 6 Pulmonalstenosen Systolischer rechter Ventrikeldruck Relative Herzgröße (V/kg) Männer ○ Frauen × Aus Reindell u Mitarb (49)

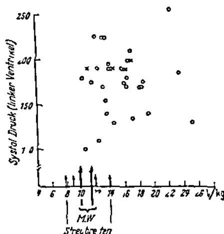


Abb 7 Aortenstenosen Systolischer linker Ventrikeldruck (Direktpunktion) Relative Herzgröße (V/kg) Männer ○ Frauen × Aus Reindell u Mitarb (49)

vermehrt druck und volumenbelasteten Herzens

Zur Beurteilung dieser Gegebenheiten haben wir die Beziehungen zwischen intrakardialen und intrapulmonalen Druckwerten absoluter und relativer Herzgrösse sowie Leistungsfähigkeit bei rechts- sowie linksseitig vermehrt druck und volumenbelasteten Herzen untersucht Als Beispiel der vermehrten Druckbelastung führen wir die Befunde der Aorten und Pulmonalstenose als Beispiel der vermehrten Volumenbelastung den Ductus Botalli die Septumdefekte und die Aorteninsuffizienz an

I *Das druckuberlastete Herz*

1 *Ergebnisse* In Abb 6 finden sich die Werte für den systolischen Ventrikeldruck und die relative Herzgrosse von 45 Pulmonalstenosepatienten Die Abbildung zeigt dass der rechte Ventrikel systolisch Drucke bis über 200 mm Hg überwinden kann, ohne dass das Herz vergrossert ist Von den insgesamt 45 Herzen ist nur ein geringer Teil vergrossert und liegt ausserhalb des oberen Normbereiches Etwa 25 % der Herzen liegt sogar im unteren Normbereich

Abb 7 enthalt die Druck Volumenbeziehungen bei 41 Patienten mit Aortenstenose (der Druck im linken Ventrikel wurde durch Direktpunktion (Doz Dr Steim) gewonnen) Auch hier ist keine Beziehung zwischen Herzgrösse und systolischer Druckbelastung festzustellen

Untersucht man die Grossenverhaltnisse des relativen Herzvolumens (Herzvolumen pro Korperoberflache), wie dies in Abb 8 fur verschiedene Formen druckbelasteter Vitien geschehen ist so ergeben sich bei insgesamt 46 Patienten in 31 Fallen eine noch normale und nur bei 15 Fallen eine pathologische Herzgrosse

Abb 9 enthalt die Beziehungen

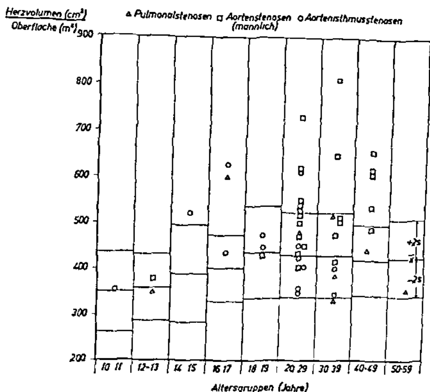


Abb 8 Die relative Herzgröße (Herzvolumen/Körperoberfläche) bei Patienten mit Pulmonalstenose, und Aortenstenose und Aortenisthmusstenose bezogen auf die dem Alter entsprechenden Normstreubereiche des Quotienten Herzvolumen/Körperoberfläche ($\bar{x} \pm 2$ s) Aus Reindell König und Mitarb (53)

zwischen dem angiographisch bestimmten Restblut des linken Ventrikels und dem *Herzvolumen bei 35 Patienten mit* Aortenstenose (Die Grossenbestimmung *des systolischen und diastolischen Restblutes* des linken Ventrikels erfolgte durch Emmrich, Reindell und Mitarbeiter (7) in Anlehnung an eine Methode *von Arvidsson (1) und Leven und* Mitarbeiter (33 a)) Aus der Abbildung *ist deutlich ersichtlich, daß die Menge* des Restblutes des linken Ventrikels bei

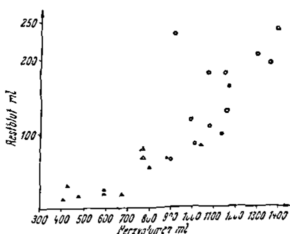


Abb 9 Herzgroße und Restblut bei Patienten mit Aortenstenose Aus Emmrich u Mitarb (7)

▲ Jugendliche bis 20 Jahren mit angeborenem Aortenvitium
● Erwachsene mit angeborenem Aortenvitium
△ Jugendliche bis 20 Jahren mit erworbenem Aortenvitium
○ Erwachsene mit erworbenem Aortenvitium

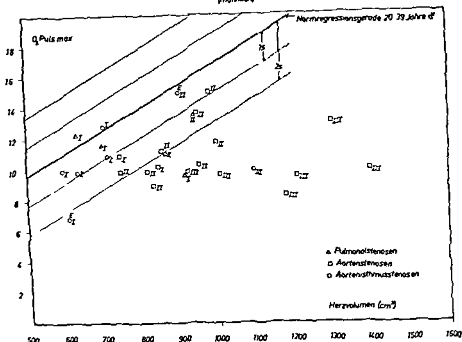


Abb 10 Die Beziehungen zwischen Herzvolumen und maximalem Sauerstoffpuls (Ergostase) bei Patienten mit Pulmonalstenose Aortenstenose und Aortenisthmusstenose bezogen auf die Streubereiche (± 2 s) der dem Alter entsprechender Normregression Aus Reindell Konig und Mutarb (53)

Patienten mit Aortenstenose deren Herzvolumen klein ist oder im oberen Normbereich liegt, klein ist

Die Leistungsfahigkeit ist bei einem Teil dieser Herzen — es wurde nicht das Gesamtgut untersucht — noch normal 53) Als Beispiel wurde ein Kollektiv 20—39 jahriger Patienten mit vermehrter Druckbelastung ausgewahlt bei denen die Beziehungen zwischen Herzvolumen und maximalem O Puls untersucht wurden (28 Falle Abb 10) (Die romischen Ziffern die jedem Meßpunkt beigegeben sind bedeuten dass das relative Herzvolumen (Herzvolumen pro kg Korperoberflache) im unteren Normbereich (I im oberen Normbereich (II) bzw ausserhalb des normalen Streubereiches (III) lag) Bei der Mehrzahl der Falle mit normal grossen relativen Herzvolumen (I und II) ist auch die Relation zwischen Herzgrosse und Leistung der Norm entsprechend Samtliche Falle mit pathologisch vergrosserten Herzvolumen (III) zeigen eine gestörte Herzleistungsrelation

Bei 7 Pulmonalstenosen mit noch normaler Leistungsbreite und normaler Herzgrosse wurde die arterio-venose Differenz in Ruhe und unter Belastungsbedingungen untersucht und mit dem Verhalten gesunder Normalpersonen verglichen (Abb 11) Die Abbildung zeigt, dass die avD O_2 auf allen Be-

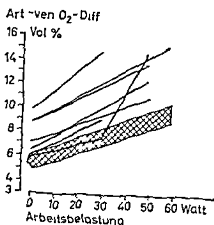


Abb. 11. Die arteriovenose O_2-Differenz in Ruhe und wahrend Belastung bei 7 Patienten mit Pulmunalstenose (ausgezogene Linien) und bei 26 gesunden Personen unterschiedlicher Leistungsbreite (4 Frauen, 13 Manner, 9 Hochleistungssportler). Das gestrichelte Feld entspricht eigenen Werten einer Serie von Normalpersonen unter gleichen Bedingungen. Aus Reindell, Konig und Mitarb. (53)

lastungsstufen vergrossert ist. Die hier vorliegende eingeschrankte Forderleistung des Herzens wurde *in Anbetracht* fehlenden Anstiegs des mittleren Vorhofdruckes als Forderungsinsuffizienz bezeichnet und von der myogenen Insuffizienz abgegrenzt.

2. *Zusammenfassung der Ergebnisse.* Es ist festzustellen, dass das menschliche Herz im Gegensatz zu den klassischen Herzgesetzen *eine pathologisch vermehrte* chronische Druckarbeit ohne Steigerung des mittleren Fullungsdruckes *und* ohne Zunahme der Restblutmenge, vielmehr mit einer Verkleinerung der Restblut*menge bewaltigt (konzentrische Hypertrophie)* (Tab. III). Die infolge der erhohten Druckbelastung verminderte Forderleistung des Herzens wird durch eine uber die Norm hinaus gehende Vergrosserung der avD O_2 kompensiert. Tritt beim druckbelasteten Herzen eine Zunahme der Restblutmenge ein, so bedeutet das muskulare Insuffizienz. Da ein druckbelastetes Herz vor der Insuffizienz nicht vergrossert ist, bei Rechtsbelastung sogar eher klein sein kann, liegt die Grosse des insuffizienten druckbelasteten Herzens nicht selten im normalen Streubereich. Es ist jedoch — *was seine Form betrifft — rechtsbetont* asymmetrisch.

II. *Das volumenuberlastete Herz*

1. *Ergebnisse.* In Abb. 12 findet sich die relative Herzgrosse (Herzvolumen/*Korperoberflache) bei 55 Patienten* mannlichen Geschlechts mit Duktus Bo-

TABELLE III. Arbeitsweise des suffizienten menschlichen Herzens bei krankhafter Druckbelastung nach den

klassischen Herzgesetzen '	neuen Herzgesetzen
Restblutvermehrung Anstieg des diastolischen Fullungsdruckes Vergrosserung des Herzens in systolischer u. diastolischer Endstellung Tonogene Dilatation (MORITZ) Widerstandsdilatation (ZDANSKY)	Restblutabnahme Kein Anstieg des diastolischen Fullungsdruckes *Verkleinerung des Herzens in* systolischer u. diastolischer Endstellung *Stadium I (Kompensation) bei* Druckbelastung. Konzentrische Hypertrophie

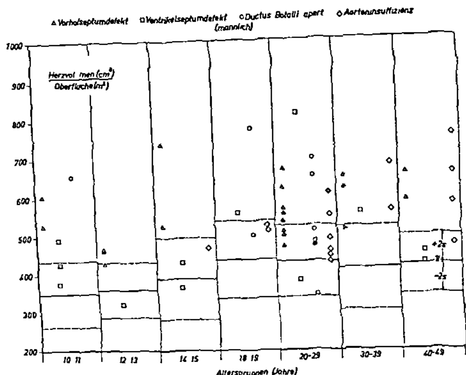


Abb 12 Die relative Herzgroße (Herzvolumen/Korperoberflache) bei Patienten mit Vorhofseptumdefekt Ventrikelseptumdefekt Ductus Botalli und Aorteninsuffizienz bezogen auf die dem Alter entsprechenden Normstraubereich des Quotienten Herzvolumen/Korperoberflache ($\bar{x} \pm 2s$) Aus Reindell Konig und Mitarb (53)

talli Vorhofseptumdefekt, Ventrikelseptumdefekt und Aorteninsuffizienz (53)

Bei insgesamt 19 Patienten mit Vorhofseptumdefekt wurde nur in 5 Fallen eine relative Herzgrösse im oberen Normbereich gefunden Alle ubrigen lagen oberhalb des Normbereiches waren also pathologisch vergrossert Dieser Befund entspricht der Feststellung von Kjellberg et al (24) der bei 77 Patienten nur 6mal eine normale Herzgrösse fand

Von 12 Patienten mit Ventrikelseptumdefekt lag bei 3 Fallen das relative Herzvolumen im unteren 2 Sigma Streubereich und 5 Falle waren dem oberen Streubereich zugeordnet Die restlichen 4 Falle waren uber die Norm vergrossert

Von 9 Patienten mit Ductus Botalli lag 1 Fall im unteren und 4 Fälle im oberen Normbereich Die restlichen 4 Falle waren uber den normalen Streubereich hinaus vergrossert

Bei 15 Patienten mit Aorteninsuffizienz lag das relative Herzvolumen in 8 Fallen noch im Normbereich, die restlichen 7 Falle waren pathologisch vergrossert.

Abb 13 zeigt eine vergleichende Betrachtung zwischen der Grösse des relativen Links Rechts-Shunts und der Gros

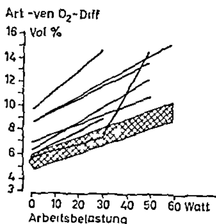


Abb. 11. Die arteriovenöse O_2-Differenz in Ruhe und während Belastung bei 7 Patienten mit Pulmonalstenose (ausgezogene Linien) und bei 26 gesunden Personen unterschiedlicher Leistungsbreite (4 Frauen, 13 Männer, 9 Hochleistungssportler). Das gestrichelte Feld entspricht eigenen Werten einer Serie von Normalpersonen unter gleichen Bedingungen. Aus Reindell, König und Mitarb. (53)

lastungsstufen vergrössert ist. Die hier vorliegende eingeschränkte Förderleistung des Herzens wurde in Anbetracht fehlenden Anstiegs des mittleren Vorhofdruckes als Förderungsinsuffizienz bezeichnet und von der myogenen Insuffizienz abgegrenzt.

2. *Zusammenfassung der Ergebnisse.* Es ist festzustellen, dass das menschliche Herz im Gegensatz zu den klassischen Herzgesetzen eine pathologisch vermehrte chronische Druckarbeit ohne Steigerung des mittleren Füllungsdruckes und ohne Zunahme der Restblutmenge, vielmehr mit einer Verkleinerung der Restblutmenge bewältigt (konzentrische Hypertrophie) (Tab. III). Die infolge der erhöhten Druckbelastung verminderte Förderleistung des Herzens wird durch eine über die Norm hinausgehende Vergrösserung der avD O_2 kompensiert. Tritt beim druckbelasteten Herzen eine Zunahme der Restblutmenge ein, so bedeutet das muskuläre Insuffizienz. Da ein druckbelastetes Herz vor der Insuffizienz nicht vergrössert ist, bei Rechtsbelastung sogar eher klein sein kann, liegt die Grösse des insuffizienten druckbelasteten Herzens nicht selten im normalen Streubereich. Es ist jedoch — was seine Form betrifft — rechtsbetont asymmetrisch.

II. *Das volumenüberlastete Herz*

1. *Ergebnisse.* In Abb. 12 findet sich die relative Herzgrösse (Herzvolumen / Körperoberfläche) bei 55 Patienten männlichen Geschlechts mit Duktus Bo-

Tabelle III. Arbeitsweise des suffizienten menschlichen Herzens bei krankhafter Druckbelastung nach den

klassischen Herzgesetzen	neuen Herzgesetzen
Restblutvermehrung Anstieg des diastolischen Füllungsdruckes Vergrösserung des Herzens in systolischer u. diastolischer Endstellung Tonogene Dilatation (Moritz) Widerstandsdilatation (Zdansky)	Restblutabnahme Kein Anstieg des diastolischen Füllungsdruckes Verkleinerung des Herzens in systolischer u. diastolischer Endstellung Stadium I (Kompensation) bei Druckbelastung: Konzentrische Hypertrophie

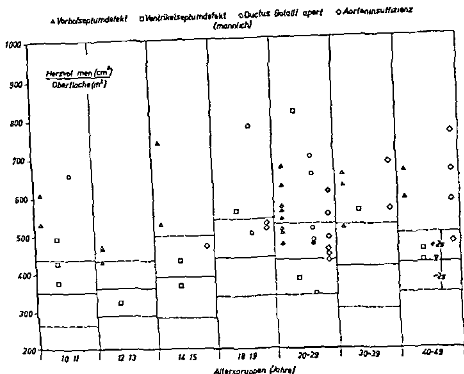


Abb 12 Die relative Herzgroße (Herzvolumen/Korperoberflache) bei Patienten mit Vorhofseptumdefekt Ventrikelseptumdefekt Ductus Botalli und Aorteninsuffizienz bezogen auf die dem Alter entsprechenden Normstraubereich des Quotienten Herzvolumen/Korperoberflache ($\bar{x} \pm 2s$) Aus Reindell Konig und Mitarb (53)

talli, Vorhofseptumdefekt, Ventrikelseptumdefekt und Aorteninsuffizienz (53)

Bei insgesamt 19 Patienten mit Vorhofseptumdefekt wurde nur in 5 Fallen eine relative Herzgrosse im oberen Normbereich gefunden Alle ubrigen lagen oberhalb des Normbereiches waren also pathologisch vergrossert Dieser Befund entspricht der Feststellung von Kjellberg et al (24) der bei 77 Patienten *nur 6mal eine normale Herzgrösse* fand

Von 12 Patienten mit Ventrikelseptumdefekt lag bei 3 Fallen das relative Herzvolumen im unteren 2 Sigma Streubereich und 5 Falle waren dem oberen Streubereich zugeordnet Die restlichen 4 Falle waren uber die Norm vergrossert

Von 9 Patienten mit Ductus Botalli lag 1 Fall im unteren und 4 Falle im oberen Normbereich Die restlichen 4 Falle waren uber den normalen Streubereich *hinaus* vergrossert

Bei 15 Patienten mit Aorteninsuffizienz lag das relative Herzvolumen in 8 *Fallen* noch im Normbereich die restlichen 7 Falle waren pathologisch vergrossert

Abb 13 zeigt eine vergleichende Betrachtung zwischen der Grosse des relativen Links-Rechts-Shunts und der Gros-

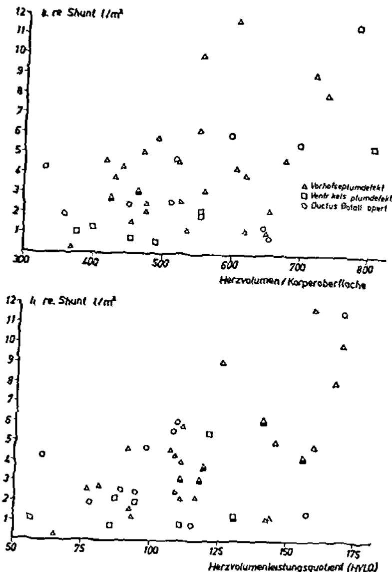


Abb 13 Korrelation zwischen relativem Shundvolumen und relativem Herzvolumen bei Patienten mit Vorhofseptumdefekt Ventrikeldefekt und Ductus Botalli Aus Reindell König und Mitarb (93)

se des relativen Herzvolumens Zwischen diesen beiden Grossen besteht eine lockere Korrelation Je grosser das Shuntvolumen, desto grosser ist im Mittel das Herzvolumen

Abb 14 enthalt die Beziehungen zwischen relativen Herzvolumen (HV / Korperoberflache) und mittleren Vorhofdruck bei 70 Patienten mit Vorhofseptumdefekt und 50 Patienten mit offenem Ductus Botalli Bei den Patienten mit Vorhofseptumdefekt wurde der mittlere Vorhofdruck von 5 mm Hg trotz teilweiser starker Herzvergrosserung nur 4 mal und bei Patienten mit Ductus Botalli nur 13 mal uberschritten Entsprechend der klassischen Herzgesetze musste aber der vermehrte Zufluss mit Fullungsdruckanstieg einhergehen

Kymographische Untersuchungen in Ruhe unter Belastungsbedingungen und unter Valsalva Einwirkung, haben er

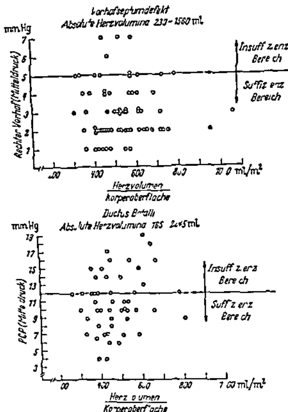


Abb. 14. Druck-Volumenbeziehungen bei angeborenen Vitien mit Volumenbelastung. Aus Reindell u. Mitarb. (49).

geben, dass das volumenüberlastete Herz in Ruhe entsprechend den klassischen Herzgesetzen nicht nur in diastolischer Endstellung — gemäss dem vermehrt zufliessenden Blut — sondern auch in systolischer Endstellung eine Grössenzunahme erfährt (56). Das bedeutet, dass das Restblut bei normalem Füllungsdruck und damit ohne Zeichen einer Insuffizienz vermehrt ist. Luthy (34) und Kreuzer (25) konnten unsere Beobachtungen mit der Thermodilutionsmethode bestätigen. Luthy fand bei kompensierter Volumenbelastung im Mittel ein Verhältnis von Schlagvolumen zu Restblut von 1 : 2.

Dieser Befund lässt sich auch sehr eindrucksvoll an Hand des postoperativen Verhaltens der Herzgrösse von Patienten mit vermehrter Volumenbelastung demonstrieren. Es wurde schon zwei Wochen nach der Operation eine diastolische Herzverkleinerung gesehen, die das Ausmass der Abnahme des Shunt-Blutes übertraf, woraus eine Verkleinerung in systolischer Endstellung resultierte.

Abb. 15 a und b zeigt links das Ky-

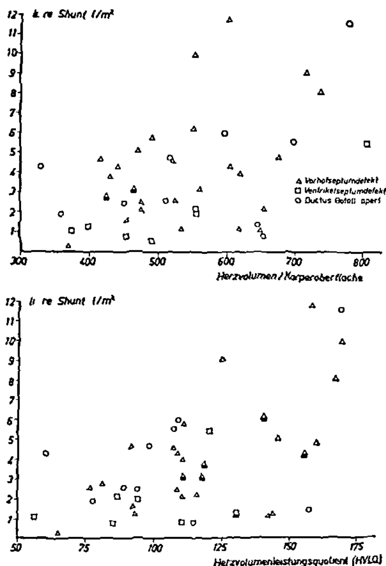


Abb 13 Korrelation zwischen relativem Shundvolumen und relativem Herzvolumen bei Patienten mit Vorhofseptumdefekt Ventrikeldefekt und Ductus Botalli Aus Reindell, Konig und Mitarb (53)

se des relativen Herzvolumens Zwischen diesen beiden Grossen besteht eine lockere Korrelation Je grosser das Shunt volumen, desto grosser ist im Mittel das Herzvolumen

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Kymographische Untersuchungen in Ruhe, unter *Belastungsbedingungen* und unter Valsalva-Einwirkung, haben er-

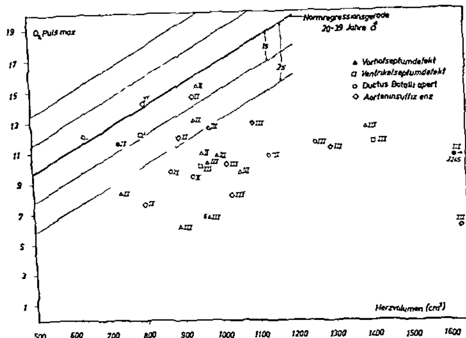


Abb. 17. Die Beziehungen zwischen Herzvolumen und maximalem Sauerstoffpuls (Ergostase) bei Patienten mit Vorhofseptumdefekt, Ventrikelseptumdefekt, Ductus Botalli und Aorteninsuffizienz bezogen auf die Streubereiche (± 2 *s*) der dem *Alter entsprechenden Normregression*. Aus Reindell, König und Mitarb. (53)

diastolisch, sondern auch systolisch eine Verkleinerung erfuhr. Beim Ductus Botalli liegen die Verhältnisse ähnlich (Abb. 16 a, b).

Abb. 17 enthält ein Teilkollektiv unseres Gesamtgutes der oben angeführten Herzfehler, in welcher das absolute Herzvolumen in Beziehung zur Leistung (O₂-Puls max) gesetzt wurde. Von 30 Fällen zeigen 8 eine normale, 22 Fälle hingegen eine pathologische Relation zwischen HV und Leistung. Man erkennt darüber hinaus, dass die Beziehungen zwischen Herzvolumen und maximalem O₂-Puls am stärksten bei denjenigen Patienten gestört sind, bei denen das Herzvolumen am grössten ist. Diese Herzvergrösserung ist Folge des Shuntvolumens, möglicherweise aber auch Folge einer zusätzlichen myogenen Dilatation (Tab. IV).

2. *Zusammenfassung der Ergebnisse*

Aus den dargelegten Befunden geht hervor, dass die klassischen Herzgesetze auch zur Deutung der Arbeitsweise von suffizienten Herzen mit vermehrter Volumenbelastung nicht herangezogen werden können.

Das vermehrt volumenbelastete suffiziente menschliche Herz ist nicht nur *diastolisch*, sondern auch systolisch vergrössert, sodass die Restblutmenge vermehrt ist, ohne dass dabei der diastolische Füllungsdruck erhöht ist. Die vergrösserte Volumenleistung dieser Herzen vollzieht sich somit in ähnlicher Weise



Abb 15 Pat F E Kymographische Untersuchung vor der Operation (a) und 14 Tage nach Verschluß des Vorhofseptumdefektes (b) Aus Reindell u Mitarb (49)

mogramm (11 11 63) einer 32-jährigen Frau mit Vorhofseptumdefekt zwei Tage vor der Operation Das Herzvolumen betrug 870 cm³ Rechts findet sich das Kymogramm 14 Tage nach der Operation Das Herzvolumen hatte sich um 255 cm³ verkleinert und betrug nur noch 615 cm³ Die vergleichende Betrachtung beider Kymogramme lässt deutlich erkennen, dass sich der rechte Vorhof und der rechte Ventrikel, der links weitgehend randbildend wurde, verkleinert hatte Darüber hinaus erkennt man, dass der rechte Ventrikel nicht nur



Abb 16 Pat Sch H Kymographische Untersuchung vor der Operation (a) und 4 Wochen nach Verschluß des Ductus Botalli (b) Aus Reindell u Mitarb (49)

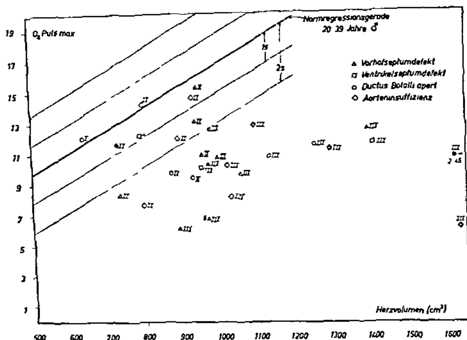


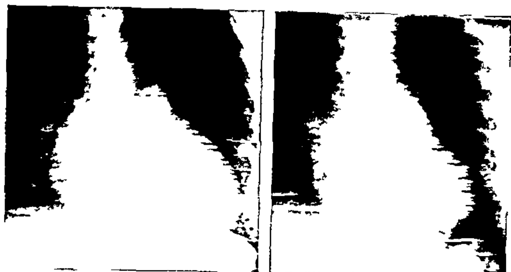
Abb 17 Die Beziehungen zwischen Herzvolumen und maximalen Sauerstoffpuls (Ergostase) bei *Patienten mit Vorhofseptumdefekt Ventrikelseptumdefekt* Ductus Botalli und Aorteninsuffizienz bezogen auf die Streubereiche (± 2 s) der dem Alter entsprechenden Normregression Aus Reindell Konig und Mitarb (53)

diastolisch sondern auch systolisch eine Verkleinerung erfuhr Beim Ductus Botalli liegen die Verhaltnisse ahnlich (Abb 16 a, b)

Abb 17 enthalt ein Teilkollektiv unseres Gesamtgutes der oben angefuhrten Herzfehler, in welcher das absolute Herzvolumen in Beziehung zur Leistung (O Puls max) gesetzt wurde Von 30 Fallen zeigen 8 eine normale, 22 Falle hingegen eine pathologische Relation zwischen HV und Leistung Man erkennt daruber hinaus dass die Beziehungen zwischen Herzvolumen und maximalem O_2-Puls am starksten bei denjenigen Patienten gestort sind bei denen das Herzvolumen am grossten ist. Diese Herzvergrosserung ist Folge des Shuntvolumens moglicherweise aber auch Folge einer *zusatzlichen myogenen Dilatation* (Tab IV)

2 *Zusammenfassung der Ergebnisse*
Aus den dargelegten Befunden geht hervor dass die klassischen Herzgesetzte auch zur Deutung der Arbeitsweise von suffizienten Herzen mit vermehrter Volumenbelastung nicht herangezogen werden konnen.

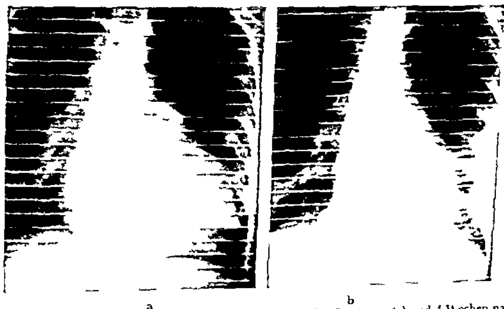
Das vermehrt volumenbelastete suffiziente menschliche Herz ist nicht nur *diastolisch sondern auch systolisch* vergrössert, sodass die Restblutmenge vermehrt ist, ohne dass dabei der diastolische Füllungsdruck erhoht ist Die vergrosserte Volumenleistung dieser Herzen vollzieht sich somit in ähnlicher Weise



a b

Abb 15 Pat F E Kymographische Untersuchung vor der Operation (a) und 14 Tage nach Verschluß des Vorhofseptumdefektes (b) Aus Reindell u Mitarb (49)

mogramm (11 11 63) einer 32 jahrigen Frau mit Vorhofseptumdefekt zwei Tage vor der Operation Das Herzvolumen betrug 870 cm³ Rechts findet sich das Kymogramm 14 Tage nach der Operation Das Herzvolumen hatte sich um 255 cm³ verkleinert und betrug nur noch 615 cm³ Die vergleichende Betrachtung beider Kymogramme lasst deutlich erkennen, dass sich der rechte Vorhof und der rechte Ventrikel, der links weitgehend randbildend wurde, verkleinert hatte *Daruber hinaus erkennt* man, dass der rechte Ventrikel nicht nur



a b

Abb 16 Pat Sch H Kymographische Untersuchung vor der Operation (a) und 4 Wochen nach Verschluß des Ductus Botalli (b) Aus Reindell u Mitarb (49)

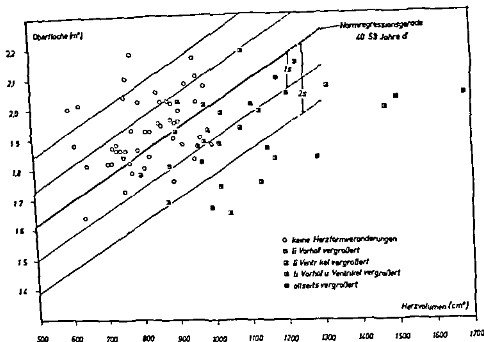


Abb 18 Die relative Herzgroße (Herzvolumen/Korperoberflache) bei Patienten mit Zustand nach komplikationslos Herzinfarkt, bezogen auf die Streubereiche (± 2 s) der dem Alter entsprechenden Normregression
(Bei allen Punkten oberhalb der Normregressionsgeraden handelt es sich um unterdurchschnittlich kleine Herzen) Aus Reindell Konig und Mitarb (53)

In der Abb 18 wurden zusatzlich die Ergebnisse einer rontgenologischen Form analyse des Herzens festgehalten Bei den mit offenen Kreisen gekennzeichne ten Fallen war die rontgenologische Herzkonfiguration normal Diese Falle zeigen vorwiegend unterdurchschnittlich kleine relative Herzvolumina Bei den ubrigen Fallen mit teilweise oder ganz ausgefullten Symbolen bestanden ront genologisch unterschiedlich stark ausge pragte pathologische Herzumformungen die den linken Vorhof den linken Vent rikel oder die beidseitigen Herzanteile betrafen Die Fälle mit pathologischen Herzumformungen sind fast durchweg dem unteren Normalbereich zugeordnet oder fallen unter den Normbereich, die le zteren sind also pathologisch vergrossert Insgesamt kann somit mit wenigen Ausnahmen in Parallelitat zum Schwere grad der Herzformveranderungen auch eine zunehmende Verschiebung der Herzvolumina in den unteren Normbe reich bzw unter den Normbereich fest gestellt werden

Aus diesen Befunden ist abzuleiten, daß die myocardialen Folgen eines Herz infarktes nicht unbedingt zu charakteristischen Großenanderungen des relativen Herzvolumens fuhren mussen Lediglich im Falle einer pathologischen Vergrosse-

TABELLE IV *Arbeitsweise des suffizienten menschlichen Herzens bei krankhafter Volumenbelastung* nach den

„Klassischen Herzgesetzen"	„Neuen Herzgesetzen"
Nur diastol. Herzvergrösserung	Diastol. u. systol. Herzvergrösserung
Keine Restblutvermehrung	Restblut*vermehrung*
Anstieg des Füllungsdruckes	*Kein* Anstieg des Füllungsdruckes
Keine Tonusänderung	*Herabgesetzer Tonus* und *erhöhte*
„tonogene Dilatation" (MORITZ)	Kontraktilität
„Zuflussdilatation" (ZDANSKY)	*Regulative* bzw. *Anpassungsdilatation*

(gemeinsam mit KLEPZIG und STEIM)

wie *beim Sportherzen* unabhängig vom Füllungsdruck. Die Größenzunahme des Herzens erfolgt somit nicht druckpassiv, sondern regulativ. Das bedeutet, dass — im Gegensatz zum druckbelasteten Herzen — eine Zunahme der Restblutmenge nicht Zeichen einer Kontraktionsinsuffizienz, sondern Ausdruck einer Anpassung ist. Unter Berücksichtigung dieser Voraussetzungen ist eine gewisse Deutung von Befunden über Herzvolumen und körperlicher Leistungsfähigkeit möglich. Bei einem noch relativ kleinen Herzen mit normaler Herzleistungsrelation ist die Aussage berechtigt, dass ein nur kleines Shuntvolumen bei suffizientem Myocard vorliegt. Dagegen ist bei stark vergrösserten Herzen und pathologischer Herzleistungskorrelation kein Rückschluss darüber möglich, ob Herzvergrösserung und Leistungsminderung ausschliesslich Folge des Shuntvolumens sind, oder aber, ob eine zusätzliche Herzmuskelschädigung mit myogener Dilatation vorliegt. Unter den letztgenannten Bedingungen wäre eine Summation von regulativer und myogener *Dilatation anzunehmen.*

Grösse und Leistungsfähigkeit des insuffizienten Herzens (Infarktherz)

Es wurde dargelegt, dass die Leistungsfähigkeit eines gesunden Herzens in einer quantitativ exakt definierbaren Relation zur Grösse des röntgenologisch bestimmten Herzvolumens steht. Die folgenden Ausführungen beschäftigen sich mit der Frage, welcher diagnostische Aussagewert dem Herzvolumen für die Beurteilung des organisch geschädigten Herzens zukommt.

1. *Ergebnisse.* Die mitgeteilten Ergebnisse gründen sich auf Untersuchungen bei Patienten mit komplikationslos abgeheiltem Herzinfarkt (26). Dieses Kollektiv wurde ausgewählt, da es sich beim Herzinfarkt um eine besonders eindeutig definierte Form einer Myocardschädigung handelt.

*Von insgesamt 78 Fällen mit Herzin*farkt ist das relative Herzvolumen bei 38 *Fällen unterdurchschnittlich* gross, bei 30 Fällen werden überdurchschnittlich *normale Herzv*olumina gefunden und 10 Fälle lassen ein pathologisch vergrösser*tes* relativ*es* Herzvolumen erkennen (Abb. 18).

Symbolen, die uber die Formverande rungen des Herzens unterrichten (vergl Abb 18), wurden mit romischen Zif fern die Gro senverhaltnisse des relati ven Herzvolumens, wie sie auch in Abbildung 17 und 19 verwendet wurden gekennzeichnet (I =oberer 2 s-Bereich II =unterer 2 s Bereich *III* = *pathologisch* vergrössert)) Von insgesamt 78 *Infarktpatienten* zeigen nur 20 Fälle eine normale Beziehung zwischen dem Herzvolumen und dem maximalen Sauerstoffpuls, es ist jedoch hervorzuhe ben dass alle diese Falle im unteren Normstreubereich liegen Die restlichen Falle weichen von der altersentsprechen den Normkorrelation ab, ihre Leistung ist also in Relation zur Herzgrosse so gering und gegenuber der Norm mehr oder minder stark eingeschrankt Alle Falle mit normaler Herzleistungsrelation weisen auch ein normales relatives Herz volumen auf (I und II) 3 Falle zeigen allerdings bereits eine linksseitige Herz umformung Unter den Fallen mit ge storter Herzleistungsrelation uberwiegen die Patienten mit pathologischer Herz konfiguration Daruber hinaus werden die starksten Abweichungen vom Norm relationsbereich bei Fallen mit vergrosserten Herzvolumina angetroffen Somit ist festzuhalten dass bei gestörter Herz leistungsrelation die meisten Falle mit noch normal grossem relativen Herzvolumen (I und II) dem *Normbereich* naher lagen als die Falle mit pathologisch vergrossertem relativen Herzvolu men (III) bei denen die Beziehungen zwischen Herzvolumen und maximalem Sauerstoffpuls die starksten Abwei chungen zeigten Die Tatsache dass auch bei normal grossen *Herzen bereits* ein Mißverhaltnis zwischen Herzgrosse und Leistung gefunden wird, bestatigt die bereits gemachte Aussage, wonach die Herzgrosse keine sicheren Hinweise uber das Bestehen einer Kontraktionsschwäche abzugeben vermag *Erst die* Verwendung eines funktionellen Korrelates, wie des maximalen Sauerstoff pulses, vermag zu einer differentialdiagnostischen Klarung zu fuhren. Ein Mißverhaltnis zwischen Herzvolumen und maximalem O_2 Puls ist Ausdruck einer Belastungsinsuffizienz, sofern extra kardiale Faktoren ausgeschlossen sind

Der Beweis fur die Richtigkeit der Annahme einer Belastungsinsuffizienz bei gestorter *Herzleistungsrelation kann* durch eine Digitalisbehandlung erbracht werden

Die folgende Abb 20 enthalt Ergebnisse uber eine 14 tagige ambulante Behandlung mit einem Digoxin Praparat bei Patienten mit komplikationslos abgeheiltem Herzinfarkt (27) Wiederum ist der Normbereich des den Patienten entsprechenden Alterskollektivs gesunder Personen zugrunde gelegt Vor der Be handlung *(offene Kreise)* liegen nahezu alle Falle unterhalb des Normbereichs und rechtfertigen damit den Verdacht auf eine Belastungsinsuffizienz. Vergleicht man die einzelnen Meßwerte vor und nach Digoxin (Pfeilrichtung), so ergeben sich aus den Veranderungen so*wohl des Herzvolumens als auch* des maximalen Sauerstoffpulses zwei wesent*liche Aussagen Fast durchweg ist* sowohl eine Verkleinerung des Herzvolu mens als auch eine Zunahme des maxi malen Sauerstoffpulses, also der Leistung festzustellen Damit war eine Verschie bung der Meßpunkte in Richtung auf

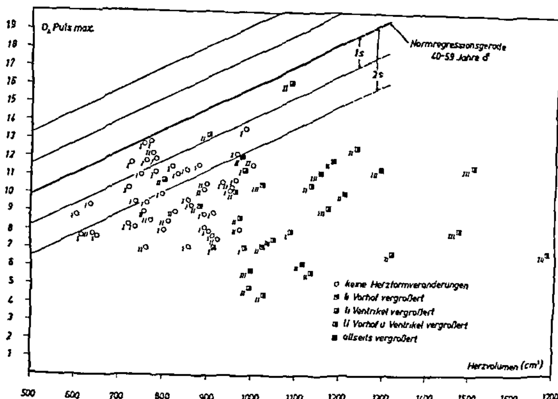


Abb 19 Die Beziehungen zwischen Herzvolumen und maximalem Sauerstoffpuls (Ergostase) bei Patienten mit Zustand nach komplikationslos abgeheiltem Herzinfarkt, bezogen auf die Streubereiche (± 2 s) der dem Alter *entsprechenden* Normregression Aus Reindell König und Mitarb (53)

rung des Herzvolumens (10 Fälle) erhält die reine Maßzahl insofern eine sichere differentialdiganostische Wertigkeit, als unter diesen Bedingungen von einer myogenen Dilatation als Ausdruck einer durch die Schädigung bedingten manifesten Kontraktionsschwäche des Herzens gesprochen werden kann Von entscheidender Bedeutung ist jedoch die Tatsache, dass die Bestimmung der Maßzahl für das absolute bzw relative Herzvolumen durch eine Analyse der röntgenologischen Herzform ergänzt werden muss Auf diese Weise kann trotz eines noch normalen Gesamtherzvolumens aus der Tatsache einer Herzumformung bzw aus deren Ausmaß auf eine bereits bestehende schädigungsbedingte Kontraktionsschwäche eines oder mehrerer Herzanteile geschlossen werden Eine solcher Art gefundene partielle oder totale Herzvergrösserung ist stets Ausdruck einer myogenen Dilatation, sofern eine Volumenbelastung ausgeschlossen werden kann

Alle bisher besprochenen grossen- und formanalytischen Betrachtungen des Herzens geben jedoch noch keinerlei Anhaltspunkte über den Kontraktionszustand bzw über die Leistungsreserven des Herzens

Die folgende Abb 19 zeigt die Korrelation der Herzgrösse mit dem Sauerstoffpuls bei diesen Herzen im Vergleich zum Normbereich 40—59jähriger gesunder Männer (Ausser den verschiedenen

st die Annahme einer Belastungs nsuffi zienz gerechtfert gt den Ausschluss einer extrakard al bed ngten Leistungsminde rung wie etwa eine zusatzl che Regula t onsstorung vorausgesetzt

D e Annahme einer Belastungs nsuffi z enz bei gestorter Herzleistungsrelation lasst s ch durch das Ergebnis e ner D gi tal sbehandlung bewe sen.

Voraussetzung einer solchen Interpre tat on ist jedoch daß d e klassischen Herzgesetze durch neue Cesetze der Herzdynam k erganzt bzw auch teil weise ersetzt v erden D ese neuen Herz gesetzte v urden im e nzelnen dargelegt

Zusammenfassung

Der absolute Wert der Herzgroße erg bt alle n ke n Urte l uber den Funkt ons- zustand des Herzens unter phys ologi schen und patholog schen Arbe tsbed n gungen Be der rontgenologischen Beur teilung eines Herzens st neben der Er kennung patholog scher Herz und Kreis- lauf erl altn sse (Vitien arterio- enose Shunts extracard ale Druckbelastung u a d e *Beurte lung se ner Leistungs- fahigke t* m H nbl ck auf den Suf f z enzgrad bez ehungsweise d e E n *schrankung der Reservekraft erwunscht* D ese erw e te ete Aussage im S nne einer funkt onellen Rontgend agnostik st erst *durch e ne korrelat e Inbeziehungset* zung des Herzvolumens zu anderen mor phologi chen und funkt onellen Korper *faktoren mogl ch* Der Vergle ch des Herzvolumens zum Korpergewicl t und zur Korperoberflacl e ergibt schon ge s e *Mogl chke ten* der *Beu teilung* Durch d e korrelat e Betrachtung von Herzgroße und Leistung st ein quanti ta es U rte l mogl cl m e e t d e Le s ungsfal gke t des Herzens unter phys o- logische Arbe tsbed ngungen se ner G oße ent pricht und in *ewe t s e unter* pa holog scl en Arbe tsbedingungen ein geschrankt ist

Schrifttum

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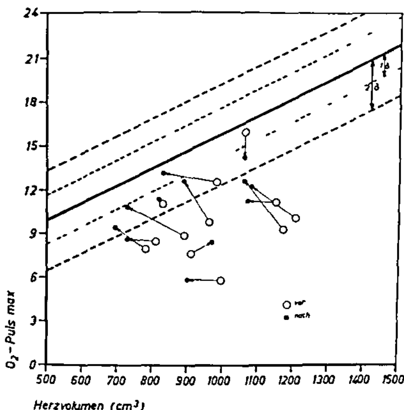


Abb 20 Die Beziehung zwischen Herzvolumen und maximalem Sauerstoffpuls bei Patienten mit Zustand nach Herzinfarkt vor und nach Digoxin Behandlung bezogen auf die Streubereiche (± 2 s) der dem Alter entsprechenden Normregression Aus Reindell, Konig und Mitarb (53)

den Normbereich bzw in den Normbereich hinein zu verzeichnen Die Belastungsinsuffizienz konnte somit durch die Glycosidbehandlung partiell oder vollig beseitigt werden

2 *Zusammenfassung der Ergebnisse* Bei einer muskularen Schadigung des Herzens tragt die korrelative Betrachtung von Herzvolumen und korperlicher Leistungsfahigkeit entscheidend zur Klarung der Frage nach dem Suffizienzgrad des Myocards bei Die Herzgrosse allein gibt nur dann eindeutige Hinweise auf den Kontraktionszustand des Myocards, wenn das relative Herzvolumen als Ausdruck einer myogenen Dilatation uber die Norm vergrossert ist. Bei der Beurteilung der Herzgrosse ist zusatzlich die rontgenologische Herzkonfiguration zu berucksichtigen Der diagnostische Aussagewert der Relation zwischen Herzgrosse und Leistung bei geschadigtem Myocard lasst sich wie folgt zusammenfassen

1 Ist die Beziehung zwischen Herzvolumen und maximalem Sauerstoffpuls normal, so kann trotz einer abgelaufenen morphologischen Herzschadigung auf eine Suffizienz des Myocards geschlossen werden

2 Ist die Beziehung zwischen Herzvolumen und maximalem Sauerstoffpuls gestort, entspricht also die Leistung nicht mehr der Grosse des Herzens so ist die Kontraktionskraft des Herzens eingeschrankt Unter diesen Bedingungen

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From Medical Department B University Hospital Oslo Norway

Hemodynamic Studies in Primary Myocardial Disease

By

O Storstein

During recent years there has been an increasing interest in primary myocardial disease This is partly due to the increased use of hemodynamic studies in obscure cardiac diseases From a hemodynamic viewpoint it is of special interest to study these diseases as the circulatory changes are brought about by a disease which solely affects the myocardium

Primary myocardial disease has been variously defined We use the definition by Mattingly (7) A cardiac disease which specifically affects the heart muscle but spares other anatomical structures within the cardiovascular system Using this classification primary myocardial disease includes myocardial disease of unknown cause as well as infiltrative processess of the myocardium like amyloidosis hemochromatosis sarcoidosis carcinomatosis progressive muscular dystrophy of the heart, fibroelastosis etc

Material and methods

During the last 6 years we have had the opportunity of carrying out hemodynamic studies in 77 patients with primary myocardial disease The final diagnosis in these patients after clinical, roentgenological, electrocardiographical, hemodynamic studies and in some patients autopsy or biopsy, is presented in Table I

As will be seen most of the patients had myocardial disease of unknown cause A few of these have died On autopsy the myocardial cells were found to be partially replaced by fibrous tissue The remaining muscle fibres were partly atrophic and partly hypertrophic In some patients there was slight infiltration of lymphocytes Even with microscopy it was difficult to tell if this was the residuum of a chronic myocarditis or if it was a primary fibrosis of the myocardium

Ten of the patients had muscular subaortic stenosis Amyloidosis of the heart was found in 6 patients scleroderma affecting the heart in 4 patients In another 4 patients with progressive muscular dystrophy there was also myo-

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From Medical Department B University Hospital, Oslo, Norway

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Ten of the patients had muscular subaortic stenosis Amyloidosis of the heart was found in 6 patients scleroderma affecting the heart in 4 patients. In another 4 patients with progressive muscular dystrophy there was also mus-

TABLE I Diagnosis in 77 patients with primary myocardial disease

Myocardial disease	44
Muscular subaortic stenosis	10
Amyloidosis	6
Scleroderma	4
Progr musc dystrophy	4
Fibroelastosis	4
Muscular hypertrophy	2
Sarcoidosis	1
Metastatic carcinoma	1
Beri beri	1
	77

cardial involvement Endocardial fibroelastosis with subendocardial infiltration of the myocardium was found in 4 patients Two patients had a peculiar disease with hypertrophy of the sceletal muscles, a hypertrophy which also involved the cardiac muscle Boecks sarcoid with involvement of myocardium was found in 1 patient One patient had cardiac affection due to carcinomatous metastases, and 1 patient a beri beri heart disease

Results and comments

Hemodynamic studies

In all of these patients hemodynamic studies have been carried out, right heart catheterization in 73 and left heart catheterization in 10 The results of right heart catheterization carried out at rest, are presented in Tables II and III

In the presentation of the findings at right heart catheterization we have divided the patients into two main groups In group 1 the patients had normal pulmonary capillary venous (PCV) pressure The upper normal limit for PCV pressure in our laboratory is 8 mm Hg, as we are using the 4th interspace in the anterior axillary line as a reference point In group 2 the patients had an elevated PCV pressure by our definition These 2 headgroups are divided into subgroups a and b, with normal and with reduced cardiac output respectively The lower normal limit for the cardiac output per square meter body surface (cardiac index) is in our laboratory 2 8

Of the 17 patients in group 1 a 10 patients had essentially normal hemodynamic findings on right heart catheterization The remaining 7 patients had slightly elevated pressure in the pulmonary artery, with normal pressures in the pulmonary capillaries and in the right atrium and slightly elevated pulmonary arteriolar resistance

The 12 patients in group 1 b had a reduced cardiac output Seven of the patients had essentially normal pressures in the lesser circulation while the remaining 5 patients had slight elevation of pulmonary arterial pressure, but normal pressures in the right and left atrium (PCV-pressure)

The 18 patients in group 2 a had slight elevation of pulmonary arterial pressure and elevated PCV-pressure Only 5 of the patients had elevation of right atrial pressure Pulmonary arteriolar resistance was mostly normal in these patients

The 20 patients in group 2 b were most severely hemodynamically affected with elevated pressures in the pulmonary capillaries and in the pulmonary artery Only 4 of the patients had a normal right atrial pressure The cardiac output was reduced and there was a moderate elevation of the pulmonary arteriolar resistance in 11 of the patients

TABLE II Hemodynamics in patients with myocardial disease with normal PCV — pressures

Age	Sex	Art O_2 satur	Cardiac index	Pulmonary artery pressure (mm Hg)	Pulmonary capillary venous pressure (mm Hg)	Right atrial mean pressure (mm Hg)	Pulmonary arteriolar resistance dynes/sec/cm⁻⁵
Group 1 a Normal cardiac output							
17	F	96	3 4	17/6	3	0	70
10	M	97	4 9	17/9	5	3	160
3	M	99	5 0	29/13	5	0	320
2	M	93	3 6	19/8	7	1	140
15	M	—	4 3	19/14	0	—1	500
2	M	98	6 4	42/12	6	0	265
29	F	93	3 8	18/9	4	0	149
37	M	98	3 9	15/6	6	1	65
34	M	94	4 4	22/7	6	2	64
15	F	98	6 7	29/12	8	4	130
20	M	96	4 5	20/8	8	0	44
17	M	96	3 6	17/5	6	2	98
43	M	98	3 8	18/5	—	2	—
58	M	98 6	3 0	40/15	5	0	315
65	M	92	3 0	36/8	—	3	265
72	F	91	3 5	28/12	—	0	—
22	M	96	3 4	16/8	—	—1	—
Mean		95 9	4 2	24/9	5 5	1	184
Group 1 b Low cardiac output							
38	M	90 3	2 5	18/5	5	0	40
29	F	95	2 7	18/10	8	4	80
36	M	95	2 7	18/4	5	0	80
23	F	95	2 2	12/5	5	2	67
24	F	95	2 2	12/5	5	2	67
21	F	97	2 1	12/4	5	1	71
20	M	98	2 3	22/6	8	2	34
67	F	97 2	1 5	24 8	5	—2	190
49	F	95	2 3	35/12	—	4 5	—
52	F	90 7	2 2	32/10	3	0	365
61	M	91	2 6	21/8	3	1	167
36	M	99	2 1	16/3	1	1	260
Mean		94	2 3	20/7	5	1 5	129

The arterial oxygen saturation was normal in the first 3 subgroups while it was slightly reduced in 4 of the patients in group 2 b

The various final diagnosis were randomly distributed in the four hemodynamic groups (1 a b, 2 a b)

There has been 2 concepts of myo-

TABLE III Hemodynamics in patients with myocardial disease with elevated PCV — pressures

Age	Sex	Art O_2 satur	Cardiac index	Pulmonary artery pressure (*mm Hg*)	Pulmonary capillary venous pressure (mm Hg)	Right atrial mean pressure (mm Hg)	Pulmonary arteriolar resistance dynes sec/cm [5]
Group 2 a Normal cardiac output							
18	M	98	4 0	24/12	11	3	53
13	M	92	4 6	26/12	10	0	193
22	M	96	3 0	17/12	10	0	80
28	F	93	5 9	29/15	13	0	42
13	M	97	3 7	22/12	12	0	42
19	F	91	4 3	36/12	15	2	137
20	F	98	3 3	23/9	11	3	68
42	F	96	4 5	58/26	16	8	236
42	M	94	3 2	52/19	21	8	133
44	M	94	3 0	28/13	14	6	135
48	F	92	4 7	30/7	18	2	123
40	F	95	3 5	38/9	13	2	56
45	M	95	3 6	30/7	9	3	89
44	M	94	3 0	28/13	14	6	135
56	M	95	3 8	32/11	12	3	40
46	F	96	5 2	52/18	15	4	139
40	M	94	3 0	50/20	22	4	192
52	M	92	3 3	40/20	18	9	160
Mean		94	3 9	35/14	14	5	114
Group 2 b Low cardiac output							
30	F	97	2 4	28/5	13	8	94
30	M	98	2 5	58/23	31	5	130
25	M	97	2 3	46/14	20	5	95
39	F	98	2 3	21/7	10	5	82
46	M	94	2 6	55/30	25	6	300
44	F	87	1 9	47/20	28	13	138
44	F	94	2 0	46/15	19	1 5	190
65	M	89	2 9	48/29	26	7	182
43	M	96	2 5	73/35	37	9	196
75	M	89 5	1 8	43/10	—	10	—
51	M	97 7	1 6	33/20	19	8	280
44	M	97	1 7	23/14	13	7	125
52	M	91	1 6	50/18	21	5	200
59	F	96	2 0	32/9	15	7	172
52	F	89	2 0	57/23	22	1	300
53	M	91	1 6	50/18	21	0	258
42	M	99	2 1	41/20	20	6	108
40	M	93	1 3	64/30	31	16	360
48	M	96	2 3	43/21	23	8	150
47	F	94	2 7	26/8	12	5	107
Mean		94	2 1	44/18	20	6	176

cardial failure I the heart failure is a forward failure and the hearts function as a pump is *compromised* II the heart failure is due to a back pressure effect of the failing myocardium, manifested by an elevation of the end diastolic pressure in the ventricles with *resulting* elevation of atrial pressure and elevation of the venous pressure in the systemic and lesser circulation The findings in the present study seem *to sustain both of* these views The patients in groups 1 a and 2 a were able to maintain a normal cardiac output at rest although some of them had elevated *pressures in the left* and some of them also in the right atrium The patients in group 1 b and 2 b had a reduced cardiac output irrespective *of the height of the pressure in* the left and right atrium The patients in group 2 showed elevation of PCV *pressure and some of them also of the right atrial pressure In these patients* the back pressure effect is the most dominant

The concept of forward failure may be *supported on studying the response to* exercise Exercise test has only been carried out in 2 patients during cardiac catheterization (Fig 1) In both of these patients the cardiac output failed to rise on exertion although both patients had a normal cardiac output at rest and one of them had normal resting pulmonary artery pressure On exertion both patients showed an abnormal rise in pulmonary artery pressure This abnormal response to exercise may be interpreted as being due to latent cardiac failure. These exercise studies demonstrate both an abnormal rise in pulmonary artery pressure and a subnormal rise in cardiac

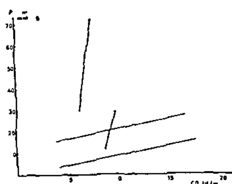


Fig 1 Response of cardiac output and mean pulmonary artery pressure to exercise test in two patients with myocardial disease compared to normal values from Holmgren & al (5)

output on exertion and illustrate both a back pressure effect and a forward failure

Various concepts have been put forward to explain the abnormal behaviour of the myocardium A *reduced compliance of the* ventricles *have been mentioned as an explanation to the myocardial* failure We feel that all consideration on myocardial compliance is mere conjecture as long as one is not able at *the same time to perform* volume and pressure measurements of the heart ventricle in man

Another explanation which has been put forward is the concept of *constrictive myocarditis* When one has had the opportunity of seeing an amyloid heart at autopsy one is impressed by the very stiff myocardium in these patients which is quite unyielding to pressure One should think that in this instance the mycardium would behave in the same way as the heart does in constrictive pericarditis where there is a failure of relaxation of the heart during diastole Study of pressure curves from patients

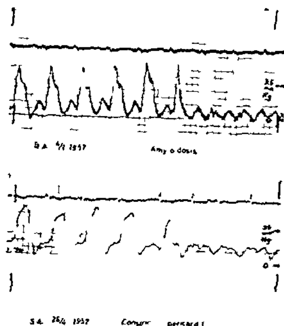


Fig 2 Pressure curves from the right ventricle and right atrium in a patient with cardiac *amyloidosis compared with pressure curves in a* case of constrictive pericarditis

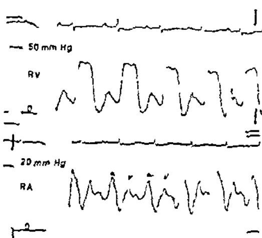


Fig 3 Pressure curves from the right ventricle (RV) and right atrium (RA) in a patient with *primary myocardial disease demonstrating an* enlarged a wave both in the right ventricle and the right atrium

with primary myocardial disease, who have elevated diastolic pressure in the right ventricle, discloses, however, that the pressure pattern in the right ventricle *is quite different from that seen in con*strictive pericarditis In the last mentioned condition there is an early diastolic dip followed by an elevated plateau during the rest of diastole In myocardial disease, on the other hand, there is also a pressure elevation in late diastole, *but here the pressure elevation coincides* with an abnormal a wave in the right atrium We feel that the concept put forward by Massumi et al (6) and also by Dye et al (3) is correct, that this diastolic pressure wave in the ventricle is a propagation of an abnormal a-wave *in the atrium* (Fig s 2 and 3) Their *studies also demonstrated that the ab*normal pressure pattern in the right *heart responded to digitalis therapy with* a diminution of the elevated a wave in the right atrium and of the elevated diastolic pressure in the right ventricle.

The differential diagnosis between constrictive pericarditis and constrictive myocarditis is very important in those cases where *there are no pericardial* calcifications on X ray In both instances there is congestive heart failure, often with considerable liver enlargement and severe venous congestion in the neck Usually there are no murmurs to be heard In both conditions there may be a diastolic heart sound In constrictive *pericarditis a pericardial clique* and *in* constrictive myocarditis *an atrial gallop* The heart is quiet in both conditions There are small pulsations on X ray and there is only a moderate cardiac enlargement One should expect the heart to be more enlarged in myocarditis than in pericarditis, but this is not a reliable sign

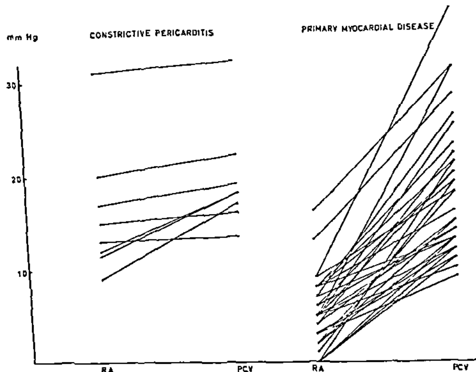


Fig 4 Comparison of mean pressures in the right atrium (RA) and pulmonary capillaries (PCV) in 8 patients with constrictive pericarditis and 37 patients with primary myocardial disease

The absence of pericardial effusion may be demonstrated in 3 ways 1) On cardiac catherization the cardiac catheter may reach the cardiac border without any interposing layer of fluid between the catheter and the cardiac border 2) By angiocardiography and 3) by injection of 50 % carbon dioxide gas

The hemodynamic studies show very much the same findings and the differential diagnosis is an enigma In our experience reliance may be placed firstly on the shape of the pressure curve in the ventricle during late diastole and secondly on the pressure niveau in the right and left atrium In myocardial disease the elevated diastolic pressure in the right ventricle is produced by the propagation of an exaggerated a wave from the right atrium, while in constrictive pericarditis the late diastolic plateau is caused by a uniform restriction to ventricular filling (Fig 2) As demonstrated by our study, the pressure is always higher in the left than in the right atrium in myocardial disease whilst in constrictive pericarditis the pressure level is almost the same in the right and left atrium (Fig 4) In some cases one has to perform thoracotomy to clarify the diagnosis as was done in 2 patients in Dye et al s series (3) Where constrictive pericarditis is found pericardial

TABLE IV Pressures in right and left heart in muscular septal hypertrophy mm Hg

Age	Sex	Date	Aorta	Sub valv	Left ventr	Pulm art	Infundibul	Right ventr	Operation
17	M	10/12/63	—	—	—	28/12	30/2	67/2	
		20/1/64	98/54	86/14	160/14	57/30	—	51/5	
		24/2/64	—	—	—	25/10	25/0	60/0	myotomia
		»				32/15	—	32/0	
24	M	10/5/61	80/60	80/10	170/0	—	—	—	
		6/9/63	—	—	—	26/10	—	41/0	
		15/10/64	108/74	108/14	188/14	—	—	—	—
43	M	25/10/63	85/60	100/10	135/12	—	—	—	
		2/12/63	—	—	—	30/10	—	30/0	exploration
43	M	27/8/63	105/75	105/6	180/4	—	—	—	myotomia
		19/11/65	130/70	130/0	205/15	—	—	—	
44	M	8/9/60	120/85	—	190/14	—	n—	—	
		19/11/65	85/60	—	175/0	—	—	—	
		13/12/65	100/70	—	120/0	—	—	—	exploration
56	F	2/2/65	110/65	—	230/0	—	—	—	myotomia
25	M	27/6/62	—	—	—	24/8	—	40/4	
		22/4/66	134/82	125/20	190/20	46/4	50/6	67/7	—
39	F	28/1/66	—	—	—	21/7	30/0	45/0	
		2/2/66	108/75	110/20	180/15	—	—	—	Propranolol
			120/75	—	120/10	—	—	—	5 mg myotomia
28	F	20/1/66	106/70	112/0	204/8	29/15	—	29/0	
			84/60	88/4	168/4	—	—	—	Propranolol 5 mg
46	F	16/11/65	120/80	—	190/5	—	—	—	
		2/2/66	120/80	—	130/0	—	—	—	exploration

decortication may be carried out If no constrictive pericarditis is found a myocardial biopsy is done Using the 2 diagnostic criteria just put forward we have not referred any patient with primary myocardial disease for operation On the other hand several patients with constrictive pericarditis without pericardial calcification have been operated on

Muscular subvalvular aortic stenosis

Muscular subaortic stenosis is a new disease described by Brock (2) Although as most other diseases this ailment was described at an earlier age by Schmincke (9)

In this interesting condition there is a hypertrophy of the muscular septum producing a subvalvular stenosis in the left ventricle and also sometimes in the right ventricle This stenosis is partly functional It is influenced by drugs (1 4)

We have had the opportunity of studying 10 of these patients The results of the pressure measurements are presented in Table IV As will be seen left heart catheterization has been carried out in all 10 patients while pressure measure

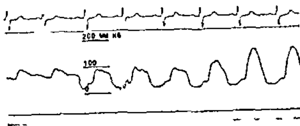


Fig 5 Pressure curve recorded during withdrawal of the catheter from the aorta to the left ventricle in a patient with muscular subvalvular aortic stenosis.

ments in the right heart has only been done in 6 of them The characteristic pressure pattern in the left heart is presented in Fig 5 As will be seen there is a low pressure in the aorta The systolic pressure in the subvalvular chamber is of the same height as that in the aorta, while the diastolic pressure in this chamber is the same as in the body of the left ventricle Here there is a high systolic pressure The systolic pressure gradient from the body of the left ventricle to the aorta varied in our patients from 50 to 220 mm Hg The stenosis may also be demonstrated on angiocardiography Fig 6 and 7 present the picture from one of our patients As will be seen there is a conical narrowing of the subvalvular chamber during systole while during diastole there is apparently normal relaxation of the chamber In 5 of the 6 cases where right heart catheterization was carried out there was a systolic pressure gradient also on the right side of the heart with similar pressure curves as from the left ventricle presenting as an infundibular pulmonic stenosis.

As we have mentioned the stenosis is partly functional. It is influenced by cardiotonic drugs. It is well known that digitalis produces an increased pressure gradient as there is a more vigorous contraction of the musculature in the left ventricle (1) In the same way beta-adrenergic stimulating agents like Isoprenol also make the stenosis more severe (1, 4) On the other hand beta adrenergic blocking agents like Propranolol may partly relieve the stenosis 1, 4)

This agent was studied in 2 of our cases In one patient 5 mg of Propranolol produced a fall both in aortic and left ventricular pressure The gradient fell slightly from 80 to 65 mm Hg The pressure effect was accompanied by a slight bradycardia In the other patient there was a more pronounced fall in left ventricular pressure from 180 to 120 mm Hg systolic The pressure in the left ventricle fell gradually over 20 minutes with a complete abolition of the pressure gradient from 70 to 0 mm Hg

Treatment of this condition is still under discussion In patients with high pressure gradients we have followed the proposal of Morrow and Brockenbrough (8) During open heart surgery the surgeon carries out an incision of the ventricular septum from the left side and makes a wedgeshaped excision of the musculature in the septum Up to now 4 of the patients have been operated on In a further 3 patients this operation was planned but after opening the heart under anesthesia and before application



Fig 6 Angıocardıogram show ıng conical narrowıng of th subvalvular chamber of the lef ventricle during systole



Fig 7 Angiocardıogram show ıng dıastolic relaxation of the subvalvular chamber

of cardio-pulmonary bypass, pressure measurement from the left ventricle and aorta showed a minimal or not existing pressure gradient We feel that this is an expression of the functional state of the muscular stenosis The diminution or abolition of the pressure gradient may be due to anesthesia producing a muscular relaxation of the hypertrophied musculature

The effect of beta adrenergic blocking agents like Propranolol seems to make it worth while to try prolonged treatment with these agents in such patients Such medical treatment is now being carried out in 2 of our patients The effect of digitalis preparations on this condition should be a warning *not to digitalize* the patients and we have accordingly stopped digitalis treatment in those patients who were under digitalis treatment

Summary

The results of hemodynamic studies in 77 patients with primary myocardial disease are presented The study seems to confirm the view that myocardial failure in these patients appears as both forward and backward failure Special emphasis is placed on the differentiation between constrictive myocarditis and constrictive pericarditis Two findings on hemodynamic studies may clarify the differential diagnosis in these patients.

1 In constrictive myocarditis the diastolic pressure in the right ventricle is elevated due to a propagated a wave from the right atrium. In constrictive pericarditis there is a uniform elevation of late diastolic pressure

2 The pressure level is always higher in the left atrium than in the right atrium in constrictive myocarditis, while in constrictive pericarditis the pressures in the left and right atrium are more on the same level

Included in this study are 10 patients with muscular hypertrophic subaortic stenosis The hemodynamic pattern in this condition is demonstrated and the functional nature of the stenosis is stressed by the demonstration of reduction of the systolic pressure gradient in 2 patients following a beta blocking agent, Propranolol, and by diminution or abolition of pressure gradient during general anestesia in 3 cases

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Fig 6 Angiocardiogram showing conical narrowing of the subvalvular chamber of the left ventricle during systole



Fig 7 Angiocardiogram showing diastolic relaxation of the subvalvular chamber

From the Department of Physiology Institute of Occupational Health Helsinki Finland the Department of Clinical Therapeutics University of Athens and M Geroulanos Institute Athens, Greece and from the Laboratory of Physiological Hygiene University of Minnesota Minneapolis U S.A

Effects of Aging on the Carotid Pulse in Two Finnish Populations

By

R. E Herron, A S Dontas M J Karvonen and A Keys

Cross sectional population studies (4 8 11) have indicated that the carotid pulse recorded transcutaneously, is characteristically transformed with aging Typically the upstroke in older persons begins rapidly but soon slows down abruptly much as in aortic stenosis (2, 5 12) This anacrotic slowing occurs earlier with increasing age As a result, the first shoulder of the pulse wave dis appears and the maximum amplitude, or wave peak comes later in the cycle This phenomenon is illustrated in Fig 1

The purpose of the present investiga tion was to examine five year age changes of the carotid pulse in the *same* individuals and to determine whether men from an area with a remarkably high incidence of coronary heart disease (East Finland) would show greater aging effects than those from an area (West Finland) where the incidence of coronary heart disease is lower (6 7)

At the same time the reproducibility of certain carotid pulse characteristics was studied as well as the usefulness of carotid pulse recordings, to supplement electrocardiographic and blood pressure data in epidemiological studies of cardio-vascular disease under field conditions, where many other laboratory procedures are excluded on practical grounds With a suitable extra arterial recording system it is possible to obtain carotid pulse tracings which are stable and similar to simultaneously recorded intra arterial ones (3 13) The procedure is not time-consuming and causes no dis comfort to the subject, and the equip ment is readily portable

Material

The subjects were drawn from among those taking part in a long term study of coronary heart disease in middle aged men living in two rural Finnish com munities In both places East Central and South West Finland (hereafter re ferred to as East and West Finland, respectively) most men of this age are lumberjacks or farmers or both, en gaged in lumbering in winter and farm-

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TABLE I Values of P(sec.) P/C (%) and a/b (%) at deciles of the distribution in 1959 and 1964

	Number of subjects	Deciles 1	2	3	4	5	6	7	8	9
P (sec.)										
1959										
East Finland (Group I)	92	0.09	0.10	0.12	0.14	0.17	0.20	0.22	0.23	0.25
East Finland (Group II)	85	0.09	0.10	0.11	0.12	0.14	0.18	0.21	0.23	0.25
West Finland (Group I)	67	0.09	0.10	0.11	0.12	0.13	0.17	0.19	0.21	0.23
West Finland (Group II)	70	0.09	0.10	0.11	0.12	0.14	0.18	0.20	0.22	0.23
1964										
East Finland (Group I)	92	0.10	0.12	0.16	0.19	0.20	0.22	0.23	0.24	0.25
East Finland (Group II)	85	0.10	0.12	0.16	0.19	0.21	0.22	0.24	0.25	0.25
West Finland (Group I)	67	0.08	0.10	0.11	0.12	0.19	0.21	0.22	0.24	0.25
West Finland (Group II)	70	0.08	0.10	0.11	0.12	0.16	0.20	0.21	0.22	0.24
P/C (%)										
1959										
East Finland (Group I)	92	10.6	12.4	13.7	17.0	19.9	21.9	23.3	25.2	26.9
East Finland (Group II)	85	10.3	11.7	12.7	14.4	17.9	20.7	23.9	25.7	27.5
West Finland (Group I)	67	10.5	12.3	14.2	16.3	17.1	21.1	22.5	24.6	26.0
West Finland (Group II)	70	9.9	11.0	13.0	14.7	17.8	21.6	23.1	24.2	27.0
1964										
East Finland (Group I)	92	11.6	15.1	19.3	21.4	23.3	24.7	25.6	26.8	28.6
East Finland (Group II)	85	10.6	14.3	19.6	20.9	22.7	23.8	25.0	26.3	28.6
West Finland (Group I)	67	9.5	11.5	13.2	15.7	20.8	22.6	23.9	25.8	27.3
West Finland (Group II)	70	9.4	10.9	13.6	14.9	18.0	21.3	22.6	25.0	26.3
a/b (%)										
1959										
East Finland (Group I)	92	65.6	70.9	76.1	78.8	81.2	82.8	86.3	90.9	92.2
East Finland (Group II)	85	61.6	69.8	73.6	76.0	79.1	83.0	86.4	89.2	92.9
West Finland (Group I)	67	67.1	71.7	75.6	79.3	82.4	85.9	87.3	89.4	92.3
West Finland (Group II)	70	61.5	72.0	75.2	8 3	81.9	84.8	88.1	91.2	93.4
1964										
East Finland (Group I)	92	55.7	60.9	63.0	65.0	70.0	73.0	75.9	80.0	86.0
East Finland (Group II)	85	52.0	56.0	59.0	63.0	70.0	73.0	78.0	81.0	86.0
West Finland (Group I)	67	59.1	62.7	65.7	67.6	71.9	75.6	78.5	82.4	89.4
West Finland (Group II)	70	62.0	64.8	68.4	70.2	72.7	76.2	78.4	80.5	85.7

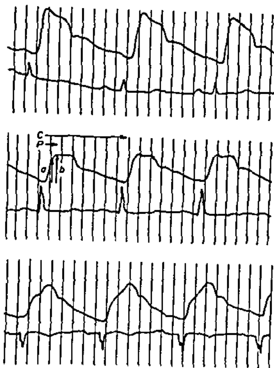


Fig 1 Typical carotid pulse contour changes with aging Reading from top to bottom, age of the subject is 41, 50 and 62 years, respectively In each case, an ECG lead was recorded simultaneously for timing purposes (lower tracing) For explanation of measurement symbols on the middle record, see section on methods (Adapted from Dontas A S, Taylor H L and Keys, A Carotid pressure plethysmograms, Effects of age, diastolic blood pressure relative body weight and physical activity *Arch Kreislaufforsch* 36 49, 1961)

ing in the summer However, intermittent periods of unemployment are common, especially in East Finland

In 1959, two out of three of all males in each region, aged 40—49 years, were randomly selected for carotid pulse recording The examination was repeated in 1964 on as many subjects as possible This resulted in 177 subjects from East Finland and 137 from West Finland being studied five years apart

Methods

The carotid pulse was recorded as described previously (3), except that a circular tambour, 4 5 cm in diameter, was used instead of a small cuff The output of the Statham P23AA pressure transducer was fed to a transistorized DC amplifier with AC coupling, having a time constant of 2 seconds at its output circuit The amplifier was connected to the DC section of an Elema Mingograph recorder The natural frequency response of the system *in situ* was 120 cycles/sec The paper speed was either 50 or 100 mm/sec and a standard ECG lead, usually V_1, was recorded simultaneously, for timing purposes, on another channel of the recorder

Recordings of the right carotid pulse were made with the subject recumbent on a hospital bed, the head being supported by a pillow This was one of a number of tests carried out during a two hour physical examination Although the period of bed rest immediately preceding the carotid recording was usually not more than five minutes, the heart rate was rarely over 100 beats/min, the majority being in the range of 60 to 80 beats/min

The following determinations were made on an average of three to five pulse cycles (1) *cycle time* (C), the time interval between successive *R* waves in the ECG, (2) *peak time* (P) the time interval between the foot and the peak of the pulse wave (3) *relative peak time* (P/C per cent) and (4) *anacrotic break* (a b per cent), where *a* is the height of the first anacrotic slowing and *b* is the maximal pulse height (Fig 1) The rationale for selecting

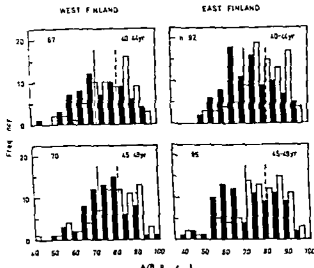


Fig 4

Figs 2 3 and 4 Frequency distributions of carotid pulse measurements P P/C and a/b in 1959 (white) and 1964 (black) The medians are represented by broken (1959) and solid (1964) vertical lines

TABLE II Mean changes in carotid pulse parameters in East and West Finland between 1959 and 1964 t values and the significance of differences

	West Finland	East Finland	t
P(s c)			
Group I	0 0151	0 0210	0 55
Group II	0 0057	0 0286	2 54[1]
P C(%)			
Group I	0 537	2 59	1 77
Group II	—0 317	2 71	2 52
a b( )			
Group I	—8 35	—10 1	0 84
Group II	—7 05	—10 0	1 31

[1] Significant at five per cent level (t = 1 97 with $N_1 + N_2 - 2$ degrees of freedom)

time (0 76) and relative peak time (0 69) In general these coefficients are lower than those obtained in 21 mental patients examined on two successive days (3) but they are within the range of the coefficients found with most electrocardiographic items (14)

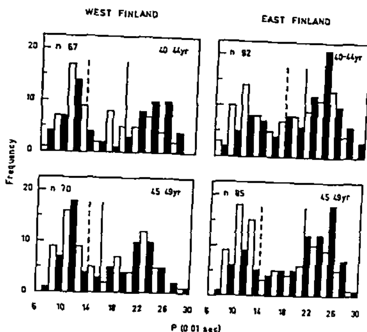


Fig 2

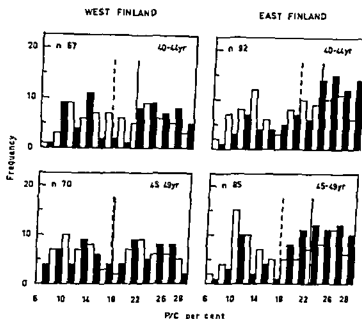


Fig 3

these particular measurements has been discussed previously in some detail (3)

In order to determine *repeat variability*, eighty subjects were examined twice in the same session in 1964 and the measurements were compared The two series of records were randomized and analyzed separately Cycle time yielded the highest reliability coefficient (0 94) followed by a b per cent (0 77), peak

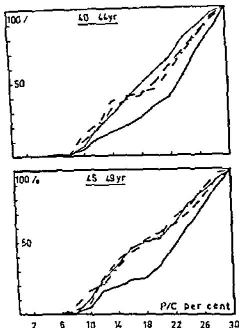


Fig 6

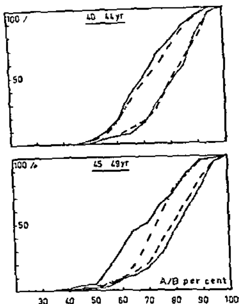


Fig 7

Figs 5 6 and 7 Cumulative percentage distributions of carotid pulse measurements P P/C and a/b used with the Kolmogorov Smirnov Test to compare 1959 and 1964 values in East and West Finland for the age ranges 40—44 (group I) and 45—49 years (Group II)

– – – 1959 East Finland ——— 1964 East Finland
1959 West Finland — — 1964 West Finland

In summary although the lapse of five years increased the P and P/C values in all four groups the effects were most marked in the older group from East Finland Although the difference in changes among the younger men from the two regions was not significant as was the case with the older men, the peak times and relative peak times in East Finland in 1964 in general typified an 'older pulse form than those in West Finland

Anacrotic break (a b per cent) In contrast to the variable changes in peak time and relative peak time changes in the relative height of the anacrotic break (a/b per cent) were more uniform (Fig 4) The decrease in the median value was almost identical in the four groups. Accordingly the mean changes for corresponding ages showed no significant differences between the two regions (Table II)

The Kolmogorov—Smirnov test indicated no significant difference in the distributions of the ratio a b per cent between the two regions in Group I, either in 1959 or 1964 However there was a significant difference between the distributions of a b ratios of the two older groups in 1964 (Fig 7) the East

Results

Peak times (P) and Relative peak times (P/C) For a more detailed analysis of age changes, the results from each region were divided into two age ranges, 40 through 44 (Group I), and 45 through 49 years (Group II) *at the time of examination in 1959*

The distribution of peak times is presented in Table I and in Fig 2 In this figure, as well as in Figs 3 and 4, each group, e g, West Finland Group I, is represented by bars, the open columns referring to 1959 results, the black columns to those obtained in 1964 on the *same* subjects Bimodality of P is demonstrated in *all* four groups, with the peak times tending to center around two modes, 0 11 and 0 23 seconds, approximately The 5-year interval brought about a general shift to the right, toward longer peak times The median values, which are indicated by broken (1959) and solid (1964) vertical lines, reflect this trend Although the direction of change was uniform, the degree of change was not In the 45—49 age range the group from East Finland showed a *significantly* larger mean change in P (Table II), but in the younger men the mean changes showed no significant differences between the two regions

When the peak time is expressed as a percentage of the cycle time, i e, as relative peak time the findings are slightly different (Fig 3), but the pattern of significant differences in mean changes remains the same (Table II) Note also that the median value of the relative peak time for Group II in West Finland was virtually the same in 1959 and 1964, and in two of the other three groups the shift of the median was less than it was for P

The Kolmogorov—Smirnov test was used to examine the hypothesis that the East Finnish subjects, from an area with higher incidence of heart disease, would show greater aging effects The cumulative percentage frequencies used in this test are given in Figs 5, 6, and 7 There was a significant difference (at the five per cent level), in the case of P and P/C, between the two populations in *1964* for both age ranges Longer time intervals were found in the East Finnish groups No significant difference between the two populations was present in 1959 for either of these measurements

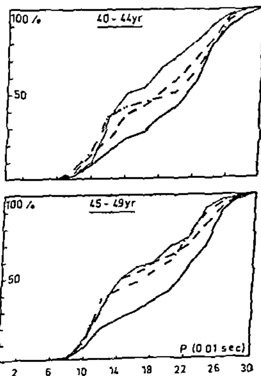


Fig 5

tained average rates of change per year of 0.9 % and 1.2 % in men of the same age from Corfu and Crete respectively

Remington (9), in a study on dogs found that the height of the anacrotic shoulder of the aortic pressure pulse was increased with myocardial stimulation and reduced with myocardial deterioration. In addition the same author pointed out that the rate of anacrotic pressure rise is clearly related to the amount of sympathetic stimulation of the left ventricle (10). He suggested that with such stimulation maximal outflow is reached earlier in systole the shoulder tends to be at a relatively high pressure level and the systolic peak of the pulse occurs earlier. On this basis, the reduction in the height of the anacrotic break and prolongation of the peak time over the five-year period in both populations might be interpreted as associated with a decrease in sympathetic stimulation or a deterioration of the contractile properties of the myocardium.

Remington's observations suggest that the anacrotic break and peak time should tend to change in parallel. The present results indicate that the two values do change in the predicted direction but not always simultaneously or in what might be considered comparable magnitudes. This is clearly demonstrated in the case of the older age group where a/b per cent changed by about the same amount in both East and West Finland but the corresponding changes in absolute and relative peak time were significantly different. Again, our measurement of "peak time" i.e. the interval from foot to either the first or the second shoulder of the pulse, could have mitigated against finding this sort of parallelism.

A brief comment about the distribution of these pulse characteristics is also in order here although distribution of peak times is definitely bimodal, that of a/b was not, with the exception of the East Finnish subjects Group II, in 1959 and in 1964. Comparison of magnitude of changes in variables distributed differently can be made only with strict reservations. Accordingly our data are presented in Table I in deciles.

A previous investigation revealed that the effect of diastolic blood pressure on the carotid pulse is similar in nature but of less importance than that of age (4). In cases of old age and elevated diastolic blood pressure there was a tendency toward "older" pulse forms. In the present sample the number of hypertensives decreased over the five-year period which reflected a general tendency toward slightly lower blood pressures. We are inclined to view this trend as an aberration in the data, either due to the original examiners being replaced in 1964 or to the subjects being more relaxed on the second occasion or both. The pulse form deteriorated in spite of the trend towards slightly lower blood pressures, and this supports the view that aging is a stronger determinant than hypertension in the morphology of the carotid pulse.

Although our main concern has been with the inter-regional carotid pulse changes over a five year period some comment on the intra-regional group differences seems pertinent. For example, Figs. 2 and 3 show that in West Fin

Finnish subjects contained generally lower values, i e, larger number of "older" type pulses

Discussion

These results indicate that measurements of certain carotid pulse characteristics can be repeated with reasonable precision Admittedly, selection of pulse cycles over which the measurements are averaged involves an element of subjectivity If there is little cycle to cycle variation, any such discrepancy between different observers is unlikely to reduce the validity of the results But from time to time distinct cycle to cycle variation does arise, usually in older individuals, which suggests that this might be a feature of clinical significance At present, we are working on a method of automatic analysis for measuring several, say twenty-five, successive pulse cycles This involves tape recording the carotid pulse, but the taping process presents no serious difficulties

During the period of five years, in men aged 40 to 49 years at the time of the first examination in 1959, greater changes in the absolute and relative peak time occurred among subjects in East Finland (Table II) There was a significant difference between the P and P/C distributions of the two populations in both age ranges, in 1964, with a greater proportion of longer time intervals in the East Finnish groups These findings of more marked age-changes in East Finland may be related to atherosclerosis and, through this common basis, to the higher prevalence of coronary heart disease in that area Moreover, a lengthening of the peak time can result from slowing of the contraction of the heart, a change known to occur in association with myocardial deterioration (15) However, in determining the *peak* of the pulse as the maximum wave amplitude, *whether this is the first or second shoulder*, our "peak time" does *not accurately reflect the rate of ejection of the heart*

The finding of a greater mean change, *towards longer peak times, in the men* from East Finland undoubtedly indicates that in more of them the wave peak has shifted from the first to the second shoulder, in the characteristic aging pattern Exactly what this represents physio*logically can not be stated at present* It might be more meaningful to measure the time intervals between the foot of the pulse and both the first and second shoulders so that their fluctuations can be studied independently This has been attempted, but presents great practical difficulties, since in the cases where one of the two shoulders is prominent, the other one may be completely obscured

The expected reduction in a b per cent with aging appeared in all four groups However, only in the older age range were the cumulative frequency distributions of this measurement regionally significantly different and then only in 1964 (East Finnish subjects showed more aging effect) The average reduction per decile in a b per cent per year was approximately the same in the two age groups of each area, but was greater in the East East Finland Group I, 2 1 %, Group II, 2 1 % West Finland Group I, 1 7 %, Group II, 1 4 % Dontas, in unpublished findings, ob

viduals and populations and can be usefully applied to epidemiological studies of cardiovascular disease

Acknowledgements

Dr Pentti Rautaharju helped us obtain the carotid pulse records and also contributed helpful advice and criticism Mr Erkki Jarvinen gave invaluable assistance with the statistical treatment of the data. To both we offer sincere thanks
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land, the men aged 40—44 in 1959 experienced a more marked shift in the median value of P and P/C than did the older group In other words, the younger group seems to have experienced an accelerated deterioration of the pulse contour, with respect to peak time and relative peak time, and outstripped the older group from the same region

Furthermore, the strikingly similar reduction in the median values of a/b ratios of all groups between 1959 and 1964, with the four medians within a range of 3 3 per cent on both occasions, indicates that in contrast to the "longitudinal" age trend, "cross sectional" age trend was non-existent, in both 1959 and 1964 surveys (Fig 4) This disparity between longitudinal and cross sectional findings is intriguing, "age trends" obtained from cross sectional data and from longitudinal studies should be distinguished

The aim of analysis of pulse contours in population studies is to obtain data relevant to central arterial mechanics, as modified by aging and heart disease Considerable progress towards a better understanding of arterial hemodynamics in general has been made in the last decade, due mainly to increased interest and improved instrumentation, but the problems are so complex that a complete understanding of the interaction of factors affecting pulse transmission is hardly likely for some time yet This is further complicated by such unknown factors, as skin and arterial wall visco-elastic changes with age Notwithstanding these difficulties serial measurements of carotid pulse contours may provide information about aging and disease of the cardio-vascular system, which can be usefully employed in evaluating the biological properties of individuals and populations In this regard, further correlational studies between the carotid pulse contour and measurements of function of other parts of the cardiovascular system in health and disease are necessary before the utility of this simple examination can be properly estimated and, where appropriate, exploited

Summary

The right carotid pulse was recorded transcutaneously with standardized technique in 314 men, aged 40—49 in 1959, and again in 1964 These men were strictly random samples of rural areas in East and West Finland which differ in frequency of coronary heart disease Recordings repeated on the same morning on 80 men gave coefficients of reliability cycle time 0 94, relative height of anacrotic break, 0 77, peak time 0 76 relative peak time 0 69

Absolute and relative peak times increased in both areas during five years, and this increase was significantly greater in East than in West Finnish men aged 45—49 in 1959 Relative height of the anacrotic break decreased in both areas but the increase in frequency of older pulse patterns was greater in East than in West Finland The follow up age trends were greater than those found in cross sectional analysis of data on the same men and were more pronounced in East Finland where the prevalence of coronary heart disease is higher

It is concluded that the carotid pulse recorded in this way can identify indi

assumption it can be shown that if the labelling is uniform then the local spreading of the tracer depot by diffusion and convection does not influence the method (15)

The fourth assumption to be made is that of stating that the recirculation can be neglected. Using an inert gas like Xenon[133] as the tracer this is the case as the elimination via the lung combined with the dilution in the whole body efficiently keeps the arterial concentration virtually at zero. For other indicators, e.g. when using non gaseous indicators with a fairly strong gamma radiation as Na^{24} or I^{131} it may be necessary to eliminate the effect of recirculation by subtracting the counting rate over a suitable non injected area from the counting rate of the injected area.

Assumptions one, two, and three imply that the local labelling achieved by injecting a tissue is equivalent to saturating the tissue completely via tracer supplied at constant concentration with the arterial blood. Assumption four implies that the onset of clearance is characterized by the abrupt drop to zero of the arterial concentration. This, therefore, constitutes the model the kinetics of which will be analyzed in the following.

Stochastic tracer theory applied to the external recording of the desaturation of a completely saturated tissue

If the tracer is neither metabolized nor retained indefinitely in a tissue then it will make a transit through the tissue following its introduction into the arterial inflow. Let the introduction of tracer be in the form of a bolus (=an "impulse") then different fractions of the bolus will have different transit times due to dispersion of the label.

The frequency function of the transit times, h(t), is defined at the site of venous outflow from the tissue (see Fig. 1). (h(t) dt) denotes that fraction of the bolus which leaves the tissue between time t and time t+dt. This implies that when adding up all the fractions then the result will be unity, i.e. the whole bolus will have been recovered. Mathematically this is expressed by integrating h(t) over all times, and hence we have $\int^{\infty} h(t)dt = \text{unity} = 1$

The mean transit time, $\bar{t}$, is given by $\bar{t} = \int^{\infty} t\ h(t)\ dt$. Defining the cumulative distribution function $H(t) = \int^{t} h(t)\ dt$, then it can be shown by integration by parts that $\bar{t} = \int^{\infty} (1 - H(t))\ dt$.

$\bar{t}$ is a very important parameter as it can be shown (8) to equal the ratio of the equilibrium volume of distribution of the tracer in the tissue under consideration, V, and its total blood flow, F

$$\bar{t} = V/F \tag{1}$$

Dividing both numerator and denominator in equation 1 by the weight of the tissue, W, and defining $\lambda = V/W$ and $f = F/W$ one obtains

$$\bar{t} = \lambda/f \tag{2}$$

λ is the equilibrium volume of distribution per gram of tissue, i.e. it is the partition coefficient between tissue and blood when taking 1 gram and 1 ml as units for the two phases respectively (7), f is the blood flow per gram of tissue.

From the Department of Clinical Physiology Bispebjerg Hospital, Copenhagen

On the Theory of the Local Clearance Method for Measurement of Blood Flow Including a Discussion of Its Application to Various Tissues

By

NIELS A LASSEN

The wash-out rate of a radioactive and freely diffusible inert tracer injected locally into a tissue was in 1949 proposed by Kety as a means of evaluating the local blood flow (6) Over the last years the method has found wide application as Xenon[133] has proved to be a very practical tracer substance to use (2, 3) This has prompted renewed considerations as to the theoretical background of the method, and recently a stochastic (probabilistic) analysis was applied to the problem resulting in a new proposal for calculating the tissue blood flow, viz that the important parameter is the relative size of *area* under the clearance curve and not its relative *slope* as believed hitherto (4) The present study also concerns the stochastic approach to the theory of the method and the analysis shows that it is the relative slope which is important This result will be discussed in relation to the more conventional Fick principle' mode of analyzing the method (7, 9) and the application of the local clearance method to various tissues will be commented on

A model of the mode of labelling of the tissue by local tracer injection

When injecting a radioactive indicator, e g Xe^{133} dissolved in 0 9% saline, locally into a tissue one is interfering directly with tissue are to be measured from One is, however, as usually in this type of considerations not taking these problems into account Indeed a steady state (linearity) of the system is assumed to exist

A second assumption is that of uniform labelling of the tissue This means that during the injection the various tissue components gets labelled in proportion to their relative volumes of distribution In other words it is assumed that neither high bloodflow areas or low-bloodflow areas gets preferentially labelled As the capillary blood is one of the tissue components this assumption is equivalent to that of stating that diffusion equilibrium between tissue and capillary blood is initially reached

The third assumption is that the tissue is isotropic i e it has the same properties in all directions Under this

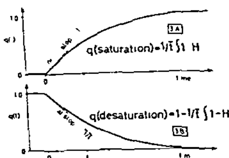


Fig 3 Stochastic theory of the local tracer injection method for measurement of tissue blood flow per unit volume of distribution (F/V or f/λ) by the relative initial slope of the externally recorded clearance curve (q(t))

3 A shows the external clearance curve q(t) recorded over tissue (=residue detection) during saturation with a constant arterial concentration The residue equals the initial concentration plus the cumulative input and minus cumulative output i e $q(t)=O+\int^t Ca-\int^t Cv=1/\bar{t}\int^t 1-H$ (the constant $1/\bar{t}$ is used in order to scale the graph so that $q(\infty)=1\ 0$ since $\int^\infty 1-H=\bar{t}$)

3 B shows the clearance curve after local tracer injection as described by the external clearance curve q(t) recorded during desaturation of a fully saturated tissue (=an uniformly labelled tissue) The curve is the mirror of 3 A i e $q(t)=1-1/\bar{t}\int^t 1-H$

In both graphs numerical value of the initial slope is $1/\bar{t}=F/V=f/\lambda$ Since the graphs are scaled so that $q(\infty)$ respectively $q(O)=1\ 0$ this initial slope is the *relative* initial slope of the actual clearance curves C(t) Thus during saturation $1/\bar{t}=(dq/dt)_{t=0}=[(dC/dt)/C(\infty)]_{t=0}$ and during desaturation $1/\bar{t}=-(dq/dt)_{t=0}=-[(dC/dt)/C(O)]_{t=0}$

integral of h(t) i e it has the form of H(t) (Fig 2 A) If the concentration is suddenly lowered to zero then by virtue of the symmetry of the situation the output takes the form of $1-H(t)$ (Fig 2 B) In order to express this desaturation curve as a frequency function, $h_{desat}(t)$ it must be normalized by dividing it by its area i e

$$h_{desat}(t)=(1-H(t))/\int^\infty(1-H(t))dt \quad (3)$$

or according to the theorem given above

$$h_{desat}(t)=(1-H(t))\ 1/\bar{t} \quad (4)$$

The third concept to be discussed is that of the *retained* amount of indicator inside the tissue q(t) This amount can be detected by external counting if the tracer is radioactive As the amount of indicator considered is always unity when using the frequency function description then q(t) is 1 minus the cumulative outflow Thus, during the desaturation described above

$$q_{desat}(t)=1-\int^t h_{desat}(t)\ dt$$

or inserting from equation (4)

$$q_{desat}(t)=1-\int^t(1-H(t))\ dt\ 1/\bar{t} \quad (5)$$

The slope of this curve at t=0 is therefore

$$[dq_{desat}(t)/dt]_{t=0}=-1/\bar{t} \quad (6)$$

This last step is correct since for all forms of h(t) we have $H(t)=\int^t h(t)dt\to 0$ for $t\to 0$ Equation 6 shows that the *initial slope* during desaturation of an *uniformly saturated tissue* (Fig 3), is a measure of the mean transit time and hence by equation 2, a measure of tissue blood flow

By external counting the counting rate is termed C(t) (or just C) and as the total amount of indicator in the tissue at time zero is unity one obtains by using equations 2 and 6

$$f=\lambda\left(\frac{-dC/dt}{C}\right)_{t=0}=\lambda\ D_0\,ml/g\ min \quad (7)$$

where D_0 is the relative slope of the externally measured clearance curve at time zero Based on the arguments advanced in the previous section the above theory of the externally recorded clearance curve after prolonged intra arterial tracer injection may be applied to the clearance of locally injected tracers

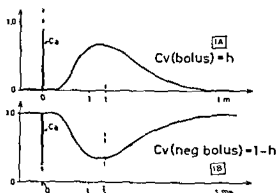


Fig 1 Outflow concentration curves, Cv, during the transit of a pulse (= bolus) of indicator The curves are scaled so that the bolus represents one unit of indicator i e so that Cv is an image of the frequency function of transit times Tissue blood flow per unit volume of distribution (F/V or f/λ) is the reciprocal of the point of gravity of the frequency function

1 A shows Cv *during* the transit of a positive pulse of indicator through a previously unlabelled tissue The venous curve is the frequency function of transit times i e Cv(pulse) = h since one unit of indicator is injected via the artery

1 B shows Cv during the transit of a negative pulse (unlabelled blood) through a previously saturated tissue The curve is the mirror image of 1 A, i e Cv(negative pulse) = 1—h

In both graphs the reciprocal of the abscissa of the point of gravity of the outflow signal is $1/\bar{t} = F/V = f/\lambda$

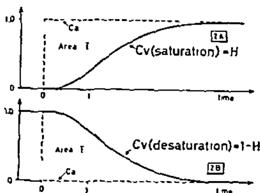


Fig 2 Outflow concentration curves, Cv, during saturation and desaturation of a tissue with a step function arterial curve Ca Tissue blood flow per unit volume of distribution (F/V or f/λ) is the reciprocal of the relative size of the area between arterial and venous curves

2 A shows Cv during saturation of a previously unlabelled tissue with a constant arterial concentration The venous curve equals the initial concentration plus the convolution of the input concentration by the frequency function of transit times i e Cv (sat) = 0 + Ca* h = H (since Ca = 1 0)

2 B shows Cv during the desaturation with unlabelled arterial blood of a completely saturated (= uniformly labelled) tissue The curve is the mirror image of 2 A i e Cv(desat) = 1—H

In both graphs the reciprocal of the area between Ca and Cv is $1/\bar{t} = F/V = f/\lambda$ Since the graphs are scaled so that Cv (∞) respectively Cv(O) = 1 0 this area is actually the *relative* size of the experimentally recorded area

The following derivation of the external recording of the desaturation of a completely saturated tissue will be made in three steps First, it may be noted that *all* tracer curves as detected in the venous outflow — also that following local injection — can be termed frequency functions of wash-out times provided the curves are normalized so that the total area is unity It is, however, solely that frequency function which follows a brief intra arterial injection which is the frequency function of *transit* times, h(t)

Second, that it is a consequence of the overall concept of a characteristic h(t) that it is constant over a certain period of some time Thus, two i a injections made one after another will simply add up in the output with the time lag given by the difference of injection times This linearity of the chance of clearance is actually the basis of the stochastic (probabilistic) theory here briefly outlined If the arterial input consist of a constant concentration it can be shown that the output now has the form of the

leads to the same solution It is of interest to note that despite the assumption here made of maintained diffusion equilibrium in each compartment then equilibrium between *the entire tissue* and its mixed venous blood is only existing initially i e uneven perfusion *manifests itself as a diffusion limitation* (because inter compartment diffusion is not assumed to be of importance) The *monocompartmental* model is a special case of the above mentioned type of analysis Consisting of only one compartment diffusion equilibrium is supposed to be maintained throughout the clearance and hence equation 7 is valid at any time (12) In this special case the blood flow need not be constant and can be calculated throughout the study from the relative slope of the clearance curve

Kety s analysis based on a constant degree of diffusion disequilibrium

The clearance curve obtained after local tracer injection was first analyzed by Kety *in 1949 (6) He assumed a con*stant ratio between the specific activity in the tissue and in the capillary blood for the label used (Na^{24}) This amounts to assuming that a constant fraction of the tracer present at any time is cleared per unit of time i e a mono exponential clearance function In this formulation the relative slope is constant and deviated from that of given by the reciprocal mean transit time ($-1/\bar{t}$) by the degree of disequilibrium Experimental evidence shows that local clearance curves are seldom *strictly* mono-exponential over several time constants (one time constant is the time the curve takes to drop to $1/e \simeq 0\ 37$ of its initial value) Usually *the relative slope decreases somewhat* with time and hence it follows from Kety s equations that it is the greatest *(initial)* slope which comes closest to diffusion equilibrium If diffusion equilibrium is assumed to exist initially then Kety s equation becomes identical to equation 7, as the arterial inflow F_a is equal to $F_{vein}+F_{lymph}$ and the term S used equals V in the present paper If diffusion equilibrium is assumed to be maintained throughout the clearance then obviously, equation 7 is valid at any time

Renkin s analysis based on the clearance concept

The subsequent theoretical considerations made by Kety in 1951 (7) on tissue clearance of tracers concerned primarily inert tracer gases and one here encounters the space of distribution per gram of tissue, λ which is the key to interpreting the slope into terms of blood flow (eq 7) These theoretical considerations combined with Renkin s clearance concept (16) forms the basis of the analysis of the local clearance method *previously published* by the present author (9)

In Renkin s terminology the Clearance Rate Cl is defined in analogy with the clearance concept in kidney physiology It is that imaginary volume of blood that would have contained the amount of indicator actually leaving 100 grams of tissue per minute if complete diffusion equilibrium had occurred. From this definition it follows that the relative slope at any time allows to measure Cl provided λ is known If

Discussion

Stochastic analysis

As mentioned in the introduction Zierler has recently as the first applied this form of analysis (20) He made the error of considering $h_{local}(t)$ as the frequency function of transit times, h(t), and hence concluded that the initial height of the curve divided by its area would equal $1/\bar{t}$ $(=\bar{f}/\bar{\lambda})$ In the special case of a strictly monoexponential clearance function $(h_{local}(t) = h(t) = 1/\bar{t}e^{-t/\bar{t}})$, then the relative initial slope (this paper) equals the height area ratio (Zierlers proposal), but, in the general case h_{local} (t) is not equal to h(t) simply because the mode of labelling differs in the venous blood following intra-arterial injection

An example may be given to make this point clearer Suppose that h(t) is a delta function (a brief "impulse") which is delayed $\bar{t}$ seconds after the intra-arterial injection In that case the clearance curve as recorded by external counting after complete saturation is a straight line reaching zero at $\bar{t}$ seconds The height area ratio hence equals $2/\bar{t}$ whereas the slope has the correct numerical value of $1/\bar{t}$ As well known, when ever a specific example can be found which disproves a general theorem then that theorem is wrong

The initial slope derivation presented in this paper does not lead to a result of direct applicability as one cannot be sure to be able to measure the initial slope accurately This modest result is actually characteristic for the stochastic approach to indicator dilution techniques in general Using it on the Stewart-Hamilton method then $\bar{t}$ is known to be the abscissa of the point of gravity (Fig 1) But, how can one be sure to be able to calculate it accurately? It depends very much on the precise recording of the longest transit times, i e on the tail of h(t) Applied to the Kety-Schmidt method the stochastic analysis shows that $\bar{t}$ is the relative size of the area (cf Fig 2) But, here again the usefulness of this knowledge depends in whether or not one can fulfill the *basic* criteria, vis a horizontal arterial curve and the precise recording of the longest transit times

The result of the stochastic analysis is nevertheless of considerable theoretical interest in all the above examples, in so far as it shows where the important parameter, $\bar{t}$, is to be looked for in the general case, i e in the case where no assumptions regarding the shape of h(t) has been made

Multi exponential analysis

The calculation of tissue blood flow from external recording of the clearance curve after complete saturation by a freely diffusible tracer supplied by the artery was described by Lassen and Ingvar (5, 11) It was assumed that the tissue in question (the cerebral cortex) was composed of *n* parallel tissue compartments each having different λ and f, each compartment maintained diffusion equilibrium throughout the clearance period The derivation showed that the initial slope was the important parameter for calculating average blood flow just as expressed in equation 7 Thus, assuming a very special form of h(t) (namely a multiexponential one)

semi logarithmic graph paper. The initial slope is steepest. The slope decreases to a fairly constant value which is reached when about 5 to 15 percent of the depot has been cleared in about 60 minutes (8). Supposedly tissue trauma (hyperemia), deposition of a part of the indicator in the cutis (reflux) and higher flow in connective tissue strands (heterogeneity) are responsible for this initial steeper curve segment. As one is wanting to study the circulation of the slowest cleared tissue component, i.e. of the adipose tissue proper, the *initial non mono exponential* part of the curve is discarded and leaving what is presumed to constitute the undisturbed fatty tissue clearance curve for analysis. Assuming that diffusion equilibrium is maintained, i.e. that uniform labelling exists of any time, then equation 7 may be used at *any time throughout the clearance pe*riod. In this manner the initial slope calculation is being used even when recording the relative slope e.g. 24 hours after the injection. One is then not making use of the steady state assumption as changes of this slope are indicative of changes of blood flow.

Skeletal muscle. In the steady state the clearance curves from skeletal muscle of Xe^{133}, Kr^{85} or I^{131} antipyrine are not strictly *mono exponential*. When about 85–95 % of the depot has been cleared then a decrease of the relative slope from its initial value is noted, i.e. a slower "tail" is seen on the curve plotted on a semi logarithmic graph paper (1,3). Linde found on repeated examinations during steady state exercise that curves with pronounced tail effect also showed slower initial clearance rate (13). Variable deposition of isotope in the subcutis (=reflux), subfascially or along connective tissue strands inside the muscle could explain this tail effect. She also found that by subtracting the slow "tail" using conventional graphical analysis the variability expressed by the standard deviation decreased. The present theory is not in contradiction with Linde's curve resolution, as her study concerns the here not described situation of imperfect labelling; indeed if one, by the subtraction, assures a more *uniform labelling of the bulk of the* depot, then the subtraction may be well justified. It should be noted, however, that one shall not necessarily strive to obtain a monoexponential first component after subtracting the slow tail part. If the first component obtained shows some curvature on the semilogarithmic plot, *then it is the initial (maximal)* slope of this component one should use for calculating muscle blood flow.

In practical use of the local clearance method it is not necessary to subtract the tail part. But, Linde's observations are of interest, as they call attention to the variability, to its probable cause and to a mode of reducing it. They also stress the basic importance of using a meticulous injection technique designed to keep tracer reflux and tissue distorsion at a minimum.

Cutaneous tissue. Preliminary studies have suggested that the hyperemic reaction of the skin following local tracer injection is so marked that it renders this mode of labelling of no value in relation to the study of the spontaneous blood flow of the cutaneous tissue (17). This has prompted the development of an

diffusion equilibrium exists at any given time then Cl equals the blood flow

Hence, if one assumes this equilibrium to prevail initially (=uniform labelling) then Renkin's clearance concept leads to equation 7

Renkin's approach is, in the opinion of the present author superior to any of the other above presented forms of analysis, as it focusses attention on the problem of diffusion equilibrium The reader is referred to a recent review for a more throughout discussion of this approach (10) The approach is just as general as the stochastic one and consequently the latter has not been presented in this paper in an attempt of improving on Renkin's theories It has been presented merely to show the result of this type of analysis and to rectify a misunderstanding

Comments on some problems encountered in relation to calculation of blood flow from the clearance curve of freely diffusible radio active tracers injected locally into a tissue

In the present paper no special diffusion properties of the indicator has been postulated Indeed it could have been labelled albumin, labelled sodium or a freely diffusible tracer as $Xenon^{133}$ the result would have been the same provided the condition stipulated was fulfilled, i e that of initial complete saturation extra — as well as intravascularly

But, in practice it is important that the indicator diffuses well across the endothelial membrane the indicator is injected into the interstitium and has to gain access to the vessels by diffusion

With labelled albumin it is presumably not possible in any tissue by local injection to approach the condition of uniform labelling This means that albumin cannot be used as tracer of blood flow It is, indeed, a tracer for lymph flow in several tissues Also radioactive ions as Na^{24} or I^{131} encounter a barrier at the capillary membrane these hydrophilic tracers are apparently restricted only to diffuse through the water-filled pores that occupy a very small fraction of the capillary wall When this restriction becomes the dominant resistance to the clearance of the ion from the interstitial space (as in hyperemic skeletal muscle in man) then their clearance rate is actually a measure of the capillary permeability (9)

The freely diffusible tracers, such as Xe^{133}, are best suited for use in the local clearance method Being lipophilic they can be assumed to encounter no diffusion barrier at the level of the cell membranes It is not the aim of this paper to discuss the important problem of the diffusion gradients of such tracers in the tissue Suffice to say that gradients must exist since label is being moved from the tissue to the capillary blood Equation 7 is therefore underestimating f to the same degree as lack of equilibrium exists One may here note that had one used the area under the clearance curve, then a further underestimation of flow would have resulted, since these curves usually have approximately a multiexponential form

Adipose tissue Injecting Xe^{133} in saline into the subcutaneous adipose tissue on the lower part of the abdomen a characteristic curve is seen when plotting on

semi logarithmic graph paper The initial slope is steepest The slope decreases to a fairly constant value which is reached when about *5 to 15 percent of* the depot has been cleared in about 60 minutes (8) Supposedly tissue trauma (hyperemia), deposition *of a part of the* indicator in the cutis (reflux) and higher flow in connective tissue strands (hetero geneity) are responsible *for this initial* steeper curve segment As one is wanting to study the circulation of the slowest cleared tissue component, i e of the adipose tissue proper the initial non mono exponential part of the curve is discarded and leaving what is presumed to constitute the undisturbed fatty tissue clearance curve for analysis Assuming *that diffusion equilibrium is maintained* i e that uniform labelling exists of any time then equation 7 may be used at *any time throughout the clearance pe riod* In this manner the initial slope calculation is being used even when re*cording the relative slope* e g 24 *hours* after the injection One is then not making use of the steady state assump*tion as changes of this slope are indica tive* of changes of blood flow

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In practical *use of the local clearance* method it is not necessary to subtract the tail part But Linde s observations are of *interest* as they call attention to the variability to its probable cause and to a mode of reducing it They also *stress the basic importance of using* a meticulous injection technique designed to keep tracer reflux and tissue distor*sion at a minimum*

Cutaneous tissue Preliminary studies have suggested that the hyperemic reaction *of the skin following local tracer injec*tion is so marked that it renders this mode of labelling of no value in relation to the study of the spontaneous *blood* flow of the cutaneous tissue (17) This has prompted the development of an

diffusion equilibrium exists at any given time then Cl equals the blood flow

Hence, if one assumes this equilibrium to prevail initially (=uniform labelling) then Renkin's clearance concept leads to equation 7

Renkin's approach is, in the opinion of the present author superior to any of the other above presented forms of analysis, as it focusses attention on the problem of diffusion equilibrium The reader is referred to a recent review for a more throughout discussion of this approach (10) The approach is just as general as the stochastic one and consequently the latter has not been presented in this paper in an attempt of improving on Renkin's theories It has been presented merely to show the result of this type of analysis and to rectify a misunderstanding

Comments on some problems encountered in relation to calculation of blood flow from the clearance curve of freely diffusible radioactive tracers injected locally into a tissue

In the present paper no special diffusion properties of the indicator has been postulated Indeed it could have been labelled albumin, labelled sodium or a freely diffusible tracer as $Xenon^{133}$ the result would have been the same provided the condition stipulated was fulfilled, i e that of initial complete saturation extra – as well as intra vascularly

But, in practice it is important that the indicator diffuses well across the endothelial membrane the indicator is injected into the interstitium and has to gain access to the vessels by diffusion

With labelled albumin it is presumably not possible in any tissue by local injection to approach the condition of uniform labelling This means that albumin cannot be used as tracer of blood flow It is, indeed, a tracer for lymph flow in several tissues Also radioactive ions as Na^{24} or I^{131} encounter a barrier at the capillary membrane these hydrophilic tracers are apparently restricted only to diffuse through the water filled pores that occupy a very small fraction of the capillary wall When this restriction becomes the dominant resistance to the clearance of the ion from the interstitial space (as in hyperemic skeletal muscle in man) then their clearance rate is actually a measure of the capillary permeability (9)

The freely diffusible tracers, such as Xe^{133}, are best suited for use in the local clearance method Being lipophilic they can be assumed to encounter no diffusion barrier at the level of the cell membranes It is not the aim of this paper to discuss the important problem of the diffusion gradients of such tracers in the tissue Suffice to say that gradients must exist since label is being moved from the tissue to the capillary blood Equation 7 is therefore underestimating f to the same degree as lack of equilibrium exists One may here note that had one used the area under the clearance curve, then a further underestimation of flow would have resulted, since these curves usually have approximately a multiexponential form

Adipose tissue Injecting Xe^{133}-in saline into the subcutaneous adipose tissue on the lower part of the abdomen a characteristic curve is seen when plotting on

Injection of Xenon 133 Lancet I 686 1964

13 LINDE, B Blood Flow in the Forearm Flexor Muscles During Exercise Studied with Iodine labelled Antipyrine Contribution to the International Symposium on Clinical Studies of Peripheral Circulation Copenhagen May 1966

14 MEIER P and ZIERLER K. L On the Theory of the Indicator-dilution Method for Measurement of Blood Flow and Volume J Appl Physiol *6* 731 1954

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epicutaneous (=transcutaneous) form of labelling by gaseous Xe^{133} (18) Due to the massive fixation of label in the underlying subcutaneous tissues a marked slowing of the clearance rate is seen after some minutes, the slope eventually (after 60—70 min) reaching the slow rate characteristic of the subcutaneous tissue This suggests that one may employ a conventional graphical analysis into two mono exponential curves and estimate the cutaneous blood flow from the initial slope of the fastest component (17)

Summary

This paper presents the application of stochastic indicator dilution to the local clearance method The analysis shows that it is the initial slope of the curve which is the important parameter to be used for calculating mean tissue blood flow

A brief description of the alternative modes of analyzing the method is also given, with particular emphasis on Renkin's clearance concept Making the same assumptions all the types of analysis gives the same result, and the stochastic analysis is only presented in order to show the result thereby obtained and to rectify a misunderstanding

Some problems encountered in the application of the clearance method for measurement of local blood flow in adipose tissue muscle and skin are briefly discussed

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From the Mayo Clinic and Mayo Foundation Section of Physiology (Dr Shepherd) Mayo Graduate School of Medicine (University of Minnesota) Rochester. Research Associate in Physiology and International Postdoctoral Fellow of the U S Public Health Service (Dr Strandell)

The Effect in Humans of Increased Sympathetic Activity on the Blood Flow to Active Muscles

By

TORE STRANDELL[1] and JOHN T SHEPHERD

During rhythmic leg exercise, the activity of the sympathetic vasoconstrictor fibers to the inactive forearm muscles is increased in proportion to the severity of the exercise (2, 5) Since it is unlikely that this increased activity would be confined to the inactive muscles, presumably there is in the active muscles a competition between the metabolic factors that dilate the muscle vessels and the increased nervous activity that tends to constrict them

In dogs (29) and cats (24) this interaction has been examined by studying the changes in vascular resistance in the hind limb muscles during their rhythmic contraction before and during stimulation of the sympathetic vasoconstrictor fibers to these muscles The results indicate that the sympathetic vasoconstrictor nerves, even when stimulated electrically to their maximum, cannot oppose the dilatation caused by the local metabolic changes in the active muscles (29) and that during steady-state conditions they cause only a minor decrease in flow (24)

The present experiments were designed to study this interaction in normal humans The blood flow to one forearm and the oxygen saturation of the venous blood from the forearm muscles were measured during and after rhythmic exercise of the forearm muscles The activity of the sympathetic vasoconstrictor fibers to the forearm muscle vessels was augmented by application of subatmospheric pressure to the lower part of the body (6)

Material

Eight healthy men aged 24 to 34 years were studied None of them were trained or accustomed to heavy arm exercise

Methods

All experiments were carried out in a temperature regulated room (21° to 22° C) Heart rate was measured from an electrocardiogram or from plethysmographic tracings of forearm blood flow Arterial blood pressure was measured

[1] Present address Clinical Physiological Laboratory Roslagstulls sjukhus Stockholm Ö Sweden

with a 20 gauge needle in the left radial artery connected to a strain gauge transducer (Statham model P23 De) and recorded with a multichannel UV recorder (Minneapolis Honeywell Visicorder 1508) Mean pressures were obtained by electrical integration

Exercise — Rhythmic exercise of the muscles of the right forearm was performed on two different hand ergometers with the subjects lying supine With the first ergometer, work was done by squeezing a water filled rubber tube (3 2 cm in diameter) between two wooden grips 60 times a minute The water was fed to a vertical glass tube, and the work was changed by varying the height that the water column was raised with each muscle contraction Because of elastic and frictional forces in the system, the work performed was not directly related to the height that the water column was raised In preliminary experiments, heights of 20 30 40, 50 60, and 70 cm were cho en as these gave a graded increase in forearm blood flow from resting to maximal or near maximal values These loads are referred to as load A, B C D E and F respectively The second ergometer was used with the idea that it might increase the likelihood that each muscle of the group of muscles involved in the exercise would do the same work with each contraction A moveable handle placed at the junction of the distal and middle phalanges was pulled toward a fixed one lying in the palm of the hand The movement of the handle was opposed by coiled metal band extension springs (Hunter Spring Co) a light bulb was illuminated when the handle was moved 3.5 cm and this was the signal for the subject to relax his muscles Because the muscles to a varying extent resisted the return of the handle, the contraction rate was set at 45/min in order to allow time for relaxation The load was varied by substituting springs of different strength Since the patterns of forearm blood flow were the same regardless of the ergometer used, the results with both ergometers have been combined for analysis

Activation of Sympathetic Vasoconstrictor Nerves — The method described by Brown *et al* (6) was used The lower part of the body was enclosed in an airtight box and sealed with a rubber sleeve ending 2 to 4 cm above the iliac crests A pressure of 60 mm Hg below ambient was achieved in the box within 5 seconds the pressure was restored to ambient more slowly (15 to 20 seconds) in order to abolish or reduce the transient reflex vasodilatation in the forearm caused by the sudden return of blood to the chest (6) Movement of the body into the box during suction was prevented by a padded harness placed between the legs For purposes of brevity this procedure will be referred to as lower body suction or suction, and situations without suction will be called control experiments

Measurement of Forearm Blood Flow — Venous occlusion plethysmography was used to measure the total forearm blood flow and xenon 133 to measure blood flow in localized regions of the forearm muscles A strain gauge plethysmograph (35) was placed over the belly of the muscles of the right forearm at the distal end of the upper quarter of the forearm In order to ensure good

drainage of the veins, the upper arm was elevated 10° to 15° and the flexed forearm positioned 25° to 30° above the horizontal plane The forearm was supported by the hand ergometer, by a small rubber pad under the medial epicondyle, and by a padded bar behind the olecranon process The changes in forearm volume were recorded on a multichannel UV recorder (Minneapolis-Honeywell Visicorder 1508) The collecting cuff was placed over the distal part of the upper arm as close to the elbow as possible and inflated to 60 to 75 mm Hg, with the cuff used, the pressure in the distal forearm veins at equilibrium was about 25 mm Hg lower than the pressure in the cuff The cuff pressure used did not measurably restrict the arterial inflow to the arm even when this was very large after severe exercise Collecting pressures between 35 to 80 mm Hg gave the same slopes of arterial inflow, whereas 95 mm Hg caused a 10 to 15 % reduction when the inflow was about 30 ml/min/100 ml and a 30 % reduction at 50 ml/min/100 ml

At rest, three to four inflow curves were recorded and averaged As it is very difficult to make measurements of flow during rhythmic exercise with plethysmography, a 5 sec rest period was inserted at different time intervals after the start of the exercise to allow the recording of one inflow curve The sequence of the number of contractions was 10 10, 20 40, 80, and 160, each followed by a 5 sec rest period The blood flows recorded between the contractions thus do not represent the average blood flow during the exercise, since the flow may be mechanically restricted during contraction (20) For convenience these flow values have been plotted against the total exercise time, and the rest periods in between have been ignored The curve obtained does not truly reflect the rate of increase in blood flow during an uninterrupted series of contractions, but it provided a convenient base line against which to assess the effect of lower body suction on the increase in blood flow with exercise After each exercise, 2 to 6 in flow curves per min were recorded for 5 to 15 min — that is, until the resting flow level again was established During the flow measurements the hand circulation was arrested by a wrist cuff inflated to 300 mm Hg The wrist cuff was inflated 1 min before resting measurements were made (22) and 20 sec before measurements during exercise, after exercise, the cuff was deflated once or twice for 1/2 to 1 min if the subject's hand was uncomfortable

The same sequence of contractions was repeated, but now lower body suction was applied after recordings had been made for 2 min at rest, recordings were then continued for 2 min before exercise was started The suction was released 3 to 8 min after exercise when blood flow had returned toward the value obtained immediately before the exercise Two control experiments and two with suction were performed at each work level, one to two work levels being completed in 2 to 4 hours

At rest and after light exercise the flow curves generally were straight and easy to interpret With increasing intensity and duration of the work load, the straight portion of the inflow curves was

shortened to two or three heart beats At the heaviest work loads, the rapid filling of the venous system on application of the collecting cuff thus became a problem This rapid filling was due to the increased flow rate combined with a decreased capacity of the veins to accom modate blood The decrease in venous capacity was related to the simultaneous increase in forearm volume This in crease could amount to 5 or 6 volumes % it was directly related to the work level had a time course similar to but somewhat slower than the flow increase and did not reach a steady state during heavy exercise These findings explain why the plethysmographic inflow curves generally were straighter after a few seconds of heavy exercise or 1 to 2 min of light exercise compared with 4 to 5 min of light exercise although the in flow rates were similar The increase in forearm volume and decrease in venous capacity may be due to continued out ward capillary filtration in exercising skeletal muscle (21) At the very end of the two heaviest loads the flows gener ally had to be calculated from the slope of the first full heart beat from either diastolic intervals or systolic peaks By simultaneous measurement of blood pressure in the median vein in two subjects it could be shown that at these highest flow rates the venous pressure increased to reach a steady value in 1 1/2 to 2 heart beats The slower volume increase thereafter at constant pressure in the median vein may be attributed to variation in filling rates in different veins delayed compliance in the venous system or outward capillary filtration Because the collection rate slowed down when venous pressure approached its equilibrium value and the measurement sometimes may have been extended into this region, the values for blood flow are probably underestimated at the heaviest work loads

Xenon 133 Clearance — Xenon 133 dissolved in sterile isotonic saline solution (Radiochemical Center Amersham, England) in a concentration of about 1 to 0 2 microcurie/ml was slowly injected with a 1 1/2 inch 30-gauge needle into the deep flexor muscles of the right forearm from the ulnar side at a depth of 2 cm the needle being withdrawn 1 4 to 1/2 min later

The disappearance rate of the isotope was measured with a 1 8-inch thick 2 inch diameter NaI (Tl) crystal coupled to a Picker digital rate computer with a linear response well above 1 000 000 counts min With 6- to 12 sec sampling periods the initial counting rates varied between 100 000 and 600 000 counts per minute The crystal was placed 4 to 5 cm from the skin the detector being re cessed 3 cm in the tubular lead col limator The curves were plotted on semilogarithmic paper, and muscle blood flow (F ml min/100 ml) was calculated as $F = \lambda \cdot K \cdot 100$ λ being the partition coefficient between tissue and blood and having the value of approximately 0 7 for skeletal muscle (11) K is the nega tive slope of the clearance curve — that is ln 2/T 1/2 when T 1/2 is the time in minutes for the activity of the depot to decrease to half (23 25)

After the xenon 133 injection, resting blood flow was measured during a 4 to 5 min period Generally, continuous exercise was started without suction for

drainage of the veins, the upper arm was elevated 10° to 15° and the flexed forearm positioned 25° to 30° above the horizontal plane The forearm was supported by the hand ergometer, by a small rubber pad under the medial epicondyle, and by a padded bar behind the olecranon process The changes in forearm volume were recorded on a multichannel UV recorder (Minneapolis-Honeywell Visicorder 1508) The collecting cuff was placed over the distal part of the upper arm as close to the elbow as possible and inflated to 60 to 75 mm Hg, with the cuff used, the pressure in the distal forearm veins at equilibrium was about 25 mm Hg lower than the pressure in the cuff The cuff pressure used did not measurably restrict the arterial inflow to the arm even when this was very large after severe exercise Collecting pressures between 35 to 80 mm Hg gave the same slopes of arterial inflow, whereas 95 mm Hg caused a 10 to 15 % reduction when the inflow was about 30 ml/min/100 ml and a 30 % reduction at 50 ml/min/100 ml

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At rest and after light exercise, the flow curves generally were straight and easy to interpret With increasing intensity and duration of the work load, the straight portion of the inflow curves was

cise Because of the difficulty of exercising the forearm muscles with the circulation of the hand arrested, the oxygen saturation values during exercise were obtained with the hand circulation free In contrast to the decrease at rest, there was no change in oxygen saturation in the deep vein when the hand circulation suddenly was arrested during light exercise in two subjects in the present series The oxygen saturation values at rest and after exercise, with the hand circulation arrested should represent blood coming from the forearm muscles (10 19, 28) Provided that the blood flow through the forearm skin and the hand is low, this may be true also for saturation values recorded during rhythmic forearm exercise even without wrist occlusion (34) although with each contraction some venous blood from the skin may drain into the deep forearm vein (12)

Statistical calculations were made according to Snedecor (33) Whenever data were averaged that included a varying number of observations per individual it was carefully checked that the average individual response was the same as the average total response

Results

Increase in Sympathetic Vasoconstrictor Activity Caused by Lower Body Suction and by Supine Leg Exercise — Both these stimuli increase the sympathetic vasoconstrictor activity to the forearm muscles (5 6) The former stimulus was chosen for the present experiment since with it there is little change in systemic arterial blood pressure Hence the changes in forearm blood flow with and without increased sympathetic activity to the forearm muscle vessels are proportional to the changes in vascular resistance Since under normal conditions the increased sympathetic activity is caused by exercise, an attempt was made to compare, in the supine subject, the intensity of the increased activity caused by rhythmic leg exercise with that caused by suction on the lower part of the body Four subjects performed leg exercise on a bicycle ergometer at a load of 820 kpm min for 10 minutes The average heart rate increased from 62 to 138 beats/min and the average systolic blood pressure, taken by auscultation from 115 to 185 mm Hg The blood flow in the resting forearm measured by xenon 133 clearance decreased from an average of 3 2 to 1 6 ml min/100 ml and later after exercise returned to 3 4 ml min 100 ml The mean decrease was 45 % (range 10 to 89 %) Judged by the increases in systolic blood pressure during exercise the mean arterial blood pressure would then have increased by approximately 20 to 30 mm Hg (3) this indicates that the vascular resistance in resting muscle had increased about 130 % — that is, about the same as the increase in the resting forearm during lower body suction

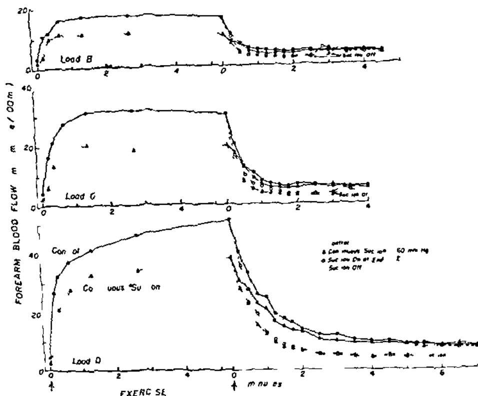
Heart Rate and Arterial Blood Pressure — The effect of forearm exercise and lower body suction on heart rate and arterial blood pressure was evaluated in the three subjects taking part in the plethysmographic study

The average heart rate increased significantly ($P < 0.01$) during exercise without suction 5 beats/min at the three

3 to 4 min followed by 3 to 4 min with suction, this sequence was then repeated Alternatively, the order was reversed and exercise was started during lower body suction for 3 to 4 min followed by 3 to 4 min of control conditions The two types of experiments with different xenon 133 injections were undertaken on the same day or in successive days in a randomized order In most subjects more than one work load was studied

The analysis of the xenon-133 clearance curves during exercise was complicated by the multiexponential decline of the activity in about three of four of the curves Some of the more obvious reasons for this soon became evident By having one-finger exercise performed at the end of the experiments, it was found that the xenon depot could be located in any one or two of the flexor muscles to the third to fifth fingers, seldom to the second finger Shifting the work load from one finger to another could increase the slope 10 fold from a resting value Even if the subject intended to keep the work load equal in all fingers, this goal was probably not reached in most instances Repeated injections deposited in different parts of the flexor muscles would be expected to give rather different flow values at the same total work level, depending on where the major part of the depot was located Whenever a depot included muscles to more than one finger, a multiexponential decline in activity could easily result, part of the activity being located in muscles with different blood flow and clearance rate If the depot happened to extend to resting muscles outside the flexor group during light exercise, a still more pronounced leveling off of the slope would be expected, the clearance rate would then approach that of the resting blood flow when the activity had left the exercising muscles Other factors responsible for multiexponential disappearance curves may of course be differences in flow rate between muscle and connective tissue in intramuscular septa and tendons

Blood Oxygen Saturation — A Teflon catheter was placed in a deep forearm vein (28), the catheter was attached to a cuvette oximeter (37) For continuous determination of oxygen saturation, blood was drawn through the catheter and oximeter by means of a motor driven syringe at a constant rate of 2 5 ml/min A correction was made for the time delay in the catheter cuvette recording assembly, which behaved like an overdamped system with one degree of freedom and a natural frequency of about 6 sec (36) For each subject the oximeter was calibrated against the Van Slyke manometric method The oxygen saturation of the deep venous blood was measured for 1 to 2 min at rest and during or after up to 10 min of exercise of the right forearm muscles During the continuous exercise a 2 1 2 to 3 min period of lower body negative pressure was applied in the middle or at the beginning and the end of the period of exercise When lower body negative pressure was applied at rest the sampling rate of 2 5 ml could not be maintained, because of the resultant decrease in forearm blood flow The hand circulation was arrested by a cuff during measurements at rest and after exer



F g 1 Effec of lower body su on on blood flow n exe c ng fo ea m lower body suc on = app cation of suba mospher pressure o he lower part of body Mean alues fo h ee young men a e gi en

s gn f cance of moderate changes of flo th suct on are to be discussed these small changes in perfu on pressure have to be cons dered

Forea m Blood Flow Measured by Plethy mog aph y — The stra n gauge was used ins ead of the con entional ater filled plethysmograph because of s con en ence Both techn ques were used n two subjects the pattern of the flow measurements after rhythm c exer cise was dent cal but the blood flo s reco ded w h the water f lled plethys mograph were 10 to 20 % lower be ca se the strain gauge neasured only o er the forearm segment that had the h ghest proport on of muscle in relat on to total volume (8)

Unless otherwise stated the results e e si ular n all three subjects n ost ly the mean values are gi en The re produc b l ty of blood flo v measure ments at rest and at the d fferent levels of exerc se a e g ven n Table I the a erage error for a s ngle determ nation in connect on w th exerc se was 7 9 % during control cond t ons and 8 4 % during lo er body suct on During control cond t ons the steady flow at the f rst compared w th the second exer

TABLE I Reproducibility of Forearm Blood Flow and Effect of Lower Body Suction (−60 mm Hg), at Rest and at Different Levels of Exercies in Three Young Men*

Work load†	Control				Suction	Decrease in blood flow with suction			
	Blood flow, ml/min/100 ml	Error			Blood flow, ml/min/100 ml			%	
		N	ml/min/100 ml	% of blood flow		N	ml/min/100 ml	Mean	SE of mean
Rest	5 3	15	0 71	13 4	3 3	30	2 0***	38	3 0
B	16 5	12	2 4	14 5	11 0	24	5 5***	33	4 9
C	31 1	9	1 6	5 2	19 6	18	11 5***	37	2 9
D	46 0	9	2 5	5 4	35 0	18	11 0***	24	2 3
E	64 5	6	4 8	7 4	58 3	12	6 2	10	5 1
F	68 8	6	4 6	6 8	57 7	12	11 1***	16	3 4

*N = number of paired observations Error = standard deviation for a single determination

† For work load B the last four exercise measurements were included, for work loads C and D the last three, and for loads E and F the last two

***$P < 0.001$, P being the probability that the differences are caused by random factors

lowest loads (B, C, D) and 13 beats/min at the two heaviest loads (E, F) During lower body suction, the average heart rate was significantly higher than during control conditions, 12 beats/min higher at rest, and 16 to 20 beats/min higher at the end of the exercise period and before the release of suction 3 to 8 min after exercise (loads B to F)

The average mean radial artery blood pressure (82 mm Hg) increased insignificantly (1 5 mm Hg) with a low work load (B) During a heavy load (E), the average mean pressure had increased by 8 mm Hg after 1/2 to 1 min exercise and by 18 mm Hg (range 11 to 20) after 2 1/2 min Within 1 1/2 min after exercise, the pressure was back to the resting value During lower body suction the average individual mean arterial blood pressure decreased by 3 5 mm Hg after 2 min at rest (range in individual experiments 0 to 7 mm Hg, n = 8), at the end of exercise (load B and E) it was 4 mm Hg lower than the control value (range 0 to 9 mm Hg, n = 9), and before the release of suction after exercise it was 6 5 mm Hg lower (range 1 to 10 mm Hg, n = 10)

The overall response to lower body suction was thus an increase in heart rate of around 15 to 20 beats min and a decrease in mean arterial blood pressure of around 5 % — that is a response similar to that observed when a subject changes from the supine to the upright position These changes in mean arterial blood pressure with suction were considered small enough so that in most instances differences in forearm blood flow with and without suction on the lower part of the body could be regarded as due to change in vascular resistance However, when the statistical

TABLE II Effect of Lower Body Suction on the Decay Rate of Blood Flow After Different Work Loads in Three Normal Men*

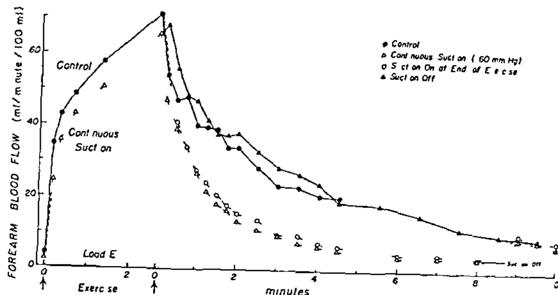
	Control		Lower body suction		Control/lower body suction	
Load	Mean sec	Range sec	Mean sec	Range sec	Mean	Range
B	16	12–19	12	8–14	1 4	1 3–1 5
C	25	23–28	16	11–23	1 7	1 0–2 2
D	50	33–66	28	25–33	1 8	1 3–2 0
E	104	75–120	44	38–50	2 4	1 7–3 1
F	125	76–180	52	30–63	3 0	1 2–6 0

* The decay rate is the time in seconds for decreasing the flow above the resting level to half and was calculated from the slope of the curve during the first 1 to 2 min after exercise

The time courses of the increase in blood flow at different work levels in these series of contractions are illustrated in Figures 1 and 2 as are the decay rates of blood flow after the end of the exercise During control conditions a steady level of blood flow was reached at the lower grades of exercise (A and B) after about 1 min total exercise time with the heavier work loads the exercise time was shorter because of fatigue (2 min 40 sec at load E 1 min 20 sec at load F) and no steady level of flow was reached With load E the average blood flow values during control conditions increased 23 % during the last 80 sec of exercise Roughly half of this increase in flow could be attributed to the corresponding increase in mean arterial blood pressure (+ 12 %), and thus only half of it was due to a decrease in vascular resistance The decay rates of the blood flow after exercise were slower after the heavier exercises compared with the lighter ones (Table II)

During lower body suction the average forearm blood flow was lower than during control conditions both at rest and at all times at the various grades of exercise and after exercise (Fig 1 and 2, Table I) The absolute difference increased from rest to exercise but the relative decrease was reduced from around 35 % at rest and at the lower levels of exercise down to 10 to 16 % at the heaviest loads It was not significant at the end of load E since one subject at this load had higher blood flow during suction than during control conditions At F the heaviest load this happened in one of six paired observations (Table I) The studies at loads E and F were combined, and the effect of suction at the end of the period of exercise was analyzed In these 12 comparisons the decrease in blood flow with lower body suction average 7 0 ml min/100 ml or 9 7 % ($P < 0\ 05$) Considering the previously observed average 5 % decrease in perfusion pressure with suction the increase in vascular resistance of about 5 % was of no significance

The effect of lower body suction on the postexercise blood flows is shown in



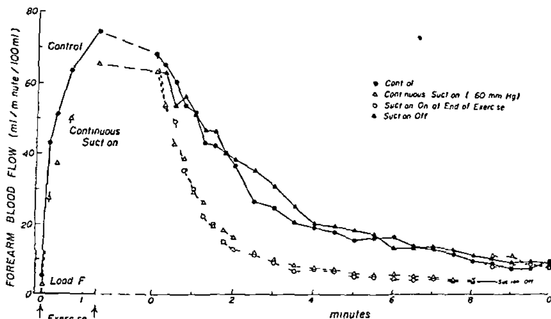


Fig 2 Effect of lower body suction on blood flow in exercising forearm Mean values for three young men are given

cise period gave a 13 % ($P<0\,01$) higher flow value at the lowest load (B) and a 4 % ($P<0\,05$) higher flow at the next higher load (C), with the other loads the differences at the end of the exercise periods were of no significance There were no systematic differences between the flow values at the first compared with those at the second exercise period with lower body suction at any work load Since the order of the experiments was randomized starting with either control conditions or lower body suction these differences did not affect the measured effect of lower body suction

TABLE II Effect of Lower Body Suction on the Decay Rate of Blood Flow After Different Work Loads in Three Normal Men*

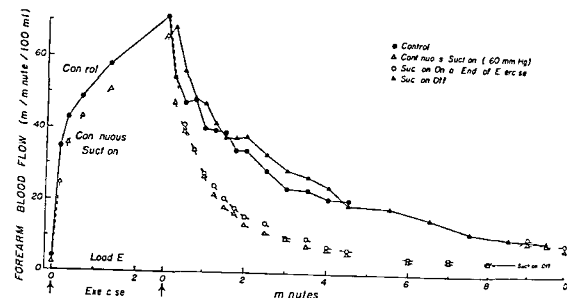
	Control		Lower body suction		Control/lower body suction	
Load	Mean sec	Range sec	Mean sec	Range sec	Mean	Range
B	16	12—19	12	8—14	1.4	1.3—1.5
C	25	23—28	16	11—23	1.7	1.0—2.2
D	50	33—66	28	25—33	1.8	1.3—2.0
E	104	75—120	44	38—50	2.4	1.7—3.1
F	125	76—180	52	30—63	3.0	1.2—6.0

* The decay rate is the time in seconds for decreasing the flow above the resting level to half and was calculated from the slope of the curve during the first 1 to 2 min after exercise

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During lower body suction the average forearm blood flow was lower than during control conditions both at rest and at all times at the various grades of exercise and after exercise (Fig 1 and 2, Table I) The absolute difference increased from rest to exercise, but the relative decrease was reduced from around 35 % at rest and at the lower levels of exercise down to 10 to 16 % at the heaviest loads It was not significant at the end of load E since one subject at this load had higher blood flow during suction than during control conditions At F the heaviest load this happened in one of six paired observations (Table I) The studies at loads E and F were combined and the effect of suction at the end of the period of exercise was analyzed In these 12 comparisons the decrease in blood flow with lower body suction average 7.0 ml min/100 ml or 9.7 % ($P < 0.05$) Considering the previously observed average 5 % decrease in perfusion pressure with suction the increase in vascular resistance of about 5 % was of no significance

The effect of lower body suction on the postexercise blood flows is shown in



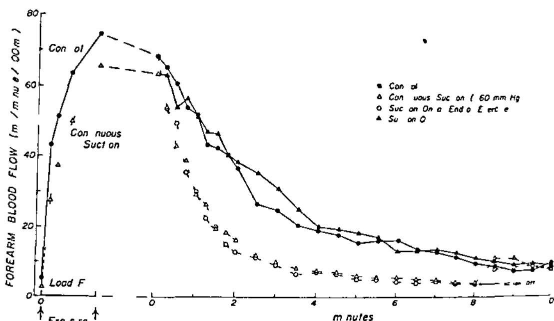


Fig 2 Effect of lower body suction on blood flow in exercising forearm Mean values for three young men are given

cise period gave a 13 % ($P<0.01$) higher flow value at the lowest load (B) and a 4 % ($P<0.05$) higher flow at the next higher load (C) with the other loads the differences at the end of the exercise periods were of no significance There were no systematic differences between the flow values at the first compared with those at the second exercise period with lower body suction at any work load Since the order of the experiments was randomized starting with either control conditions or lower body suction these differences did not affect the measured effect of lower body suction

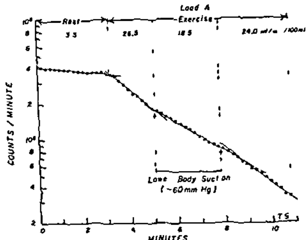


Fig 4 Effect of lower body suction on muscle blood flow calculated from clearance of xenon 133 from a 0.1 ml depot in deep forearm flexor muscles

to 2 min of the postexercise flow curves During control conditions this time increased with increasing work load When lower suction was applied in the post exercise period this time was decreased by a third at the lowest load compared with two thirds at the heaviest load

Xenon 133 Clearances From Intramuscular Depots — The effect of lower body suction on the resting forearm muscle blood flow was studied in six subjects. From an average resting flow value of 3.8 ± 1.1 ml min 100 ml (± SD n = 12) the clearance rate decreased by 56 ± 7 % (mean ± SD $P < 0.001$ n = 12) with lower body suction

A comparison between exercise blood flows calculated from xenon 133 clearances from the flexor muscles and those from plethysmographic tracings was made in three subjects The average blood flows calculated from the clearance rates at the end of 3 to 4 min of exercise were 29 ml min/100 ml (range 26.5 to 31) at load A and 57 ml min/100 ml (range 48 to 66) at load C These values were high compared with the plethysmographic flow values after the same work loads, namely 12.0 and 31 ml min/100 ml respectively This seems however very reasonable since the flexor muscles should be the ones most engaged in this type of fairly light exercise and the plethysmographic flows are an average of flow rates not only in these muscles but also in less active or even resting muscles and in the volume of bone and skin included in the forearm segment

The effect of lower body suction on the blood flow in the exercising forearm was studied in eight subjects working both on the water filled ergometer and the spring loaded ergometer The work loads used were loads A and C for the water filled one and 1.9 3.2 and 5.3 kpm min for the spring ergometer Since the effect of lower body suction was the same the data from the two ergometers were treated together

In these subjects one injection of xenon 133 was made and rhythmic exercise of the forearm muscles com

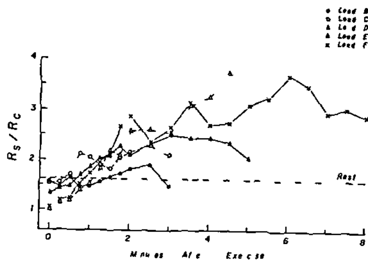


Fig 3 Ratio of forearm vascular resistance during lower body suction to resistance without suction (R_s/R_c) after different work loads Mean values for three young men are given

Figures 1 and 2 In one of the two control experiments at each work load in each individual lower body suction was applied 5 sec after the end of exercise The postexercise blood flow then decreased along the same curve as when continuous suction was applied and much quicker and to lower values than during control conditions When suction was released 3 to 8 min after exercise there was no sustained increase above the control blood flow but sometimes a slight transient increase occurred lasting 20 to 50 sec consistent with the reflex dilatation described by Brown *et al* (6) There was thus no repayment of the postexercise blood flow debt caused by the lower body suction In one of the two experiments at each work load in each individual with lower body suction during exercise the suction was released 5 sec after the end of exercise The post exercise blood flow then decreased in the same way as when no suction had been applied during exercise In other experiments it was also observed that when suction was applied or released for 2 min periods anywhere in the hyperemic postexercise period the blood flow shifted between the control curve and the curve during lower body suction

The postexercise changes in forearm vascular resistance due to lower body suction are shown in Figure 3 In the 1/2 — to 1 minute period after exercise when the blood flow was high the effect of lower body suction on vascular resistance was less pronounced than at rest Thereafter it was more pronounced and 4 to 6 min after the end of heavy exercise when control blood flow was decreased to 10 to 20 ml min 100 ml the effect on the vascular resistance was increased three to four times as compared with the effect at rest This is similar to the findings during exercise namely that the percentage decrease in flow and increase in resistance was less as the flow became very large The ability of lower body suction to increase the decay rate of blood flow after heavy compared with light exercise is illustrated in Table II Here the time for decreasing the blood flow in excess of the resting level to half was calculated from the approximately exponential decline of the first 1

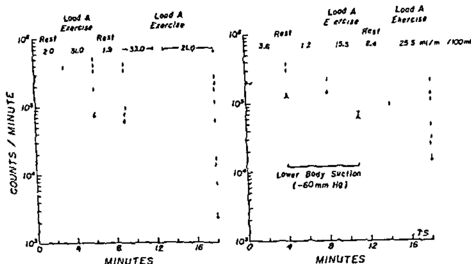


Fig 5 Effect of lower body suction on muscle blood flow calculated from clearance of xenon 133 from depots (separate days) in deep forearm flexor muscles

loads the range of blood flows without suction was 24 to 66 ml min/100 ml with a mean value of 37 4 ml/min/100 ml With lower body suction the flow values were on the average 29 % lower (range 0 to 63 % SD = 22 5 % $P < 0.001$) This difference was less marked at higher work loads and flow levels with a negative correlation coefficient between the decrease in blood flow with suction and the flow without suction ($r = -0.57$ $P < 0.05$)

In another five experiments in three subjects, the effect of lower body suction was studied by repeated exercise using the same xenon 133 depot A 3 to 4 min exercise period with lower body suction was followed after a 3 min rest period by a similar 3 to 4 min exercise period but without suction (Fig 5) In four of these experiments the calculated exercise blood flow during suction was 25 to 41 % lower than the control flow and in the fifth it was 4 % higher the average difference was 27 %

From these different modifications of experiments it seems reasonable to conclude that the significant decrease in xenon 133 clearance and calculated muscle blood flow with lower body suction was about 50 % at rest, averaged 20 to 25 % at the exercise levels used and was less marked at higher flow rates and work loads

Oxygen Saturation in Venous Blood — When lower body suction was applied during forearm exercise the oxygen saturation of the blood in a deep forearm vein decreased at all work loads used (A C D) in the three subjects tested the absolute decrease was greater at the lower work load compared with the other loads The experimental procedure and the results in one subject are given in Figure 6

Assuming an unchanged oxygen con

menced (Fig 4) When the clearance rate of xenon became constant, suction was applied for a 3- to 4-min period during the continuous exercise There was an average decrease of forearm muscle flow, calculated from the clearance value, of 32 ± 12 % (mean ± SD, $P < 0.001$, n = 12, 7 subjects) However, when suction was released during continuous exercise, there was no significant increase in clearance rate and calculated blood flow, on the average the flow values during suction were lower by 4 0 ± 12 % (mean ± SD, $P > 0.1$, n = 21, 8 subjects) There was thus a discrepancy between the effect of applying and that of releasing lower body suction during continuous exercise This seemed to be related to the fact that the clearance curves in most instances did not show a purely monoexponential decline during continuous exercise in the absence of suction but successively leveled off in varying degree although total forearm blood flow according to plethysmographic data was fairly constant Any induced decrease in clearance rate and blood flow during continuous exercise would thus be overestimated, and correspondingly any induced increase in clearance rate would be underestimated

This multiexponential decrease in activity during exercise after forearm muscle injections of antipyrine and xenon has previously been pointed out (26) and has been considered as evidence for a dual circulation in muscle. In the present study, 10 clearance curves with constant exercise for 7 to 16 min (mean 10 1/2 min) down to 4 1 % (range 1 3 to 8 7 %) of peak activity were analyzed by a double exponential-curve-fitting procedure (17) Assuming the same partition coefficient between tissue and blood in the two 'compartments," the relative weight of the first, or fast, "compartment" averaged 78 % (range 28 to 99 6 %), and the average flow of the second, or slow, "compartment" was 12 0 ml/min/100 ml (range 0 to 20 2) or 24 % (range 0 to 40 %) of the flow in the first one There was no consistent difference in this respect betwen the five curves with lower body suction and those without The large scatter in the relative weight of the fastest "compartment" and the large scatter in the blood flow in the slowest one seem to be reasonably explained by the possibility that the xenon depot spread to parts of the muscle with different activity and blood flow, as discussed in the section on Methods The actual size of an intramuscular xenon depot is not yet known, but by scintillation scanning an estimate has been made that it is less than 1 cm in diameter (7) The above-mentioned problems with the xenon-133 clearance curves during exercise certainly reduced the reproducibility and accuracy of the flow measurements, but they did not invalidate the study of the effect of lower body suction, since mostly the errors cancelled out in the comparison

In six of the eight subjects, two separate 3- to 4 min exercise periods were therefore performed at the same work load with and without lower body suction and the blood flows were calculated from the initial parts of two different xenon 133 clearance curves In 13 comparisons over a range of paired work

cise (Fig 7), when calculated arterio-venous oxygen differences were increased and blood flows were decreased approximately to half during lower body suction The same result was obtained when lower body suction was applied at the end of the exercise as when it was applied 30 sec before the exercise stopped There was no overshoot in the oxygen saturation when lower body suction was released during the postexercise hyperemia in any of the three subjects

When the changes in venous oxygen saturation are evaluated, it appears that lower body suction decreases blood flow in active muscles and increases vascular resistance when exercise is light but not significantly at heavier exercise Also the postexercise hyperemia is markedly reduced by the suction

Evaluating changes in both flow rate and venous oxygen saturation the decrease with suction in flow rate during and after exercise did not seem to delay the metabolic recovery or to cause any metabolic debt

Discussion

When humans exercise there is an increase in activity in the sympathetic adrenergic fibers that is proportional to the severity of the exercise As a consequence there is a reflex increase in wall tension of the resistance vessels in vascular beds outside the active muscles so that the blood flow to inactive muscles and abdominal organs is maintained constant or decreased in spite of the increase in systemic arterial blood pressure (2 5 31

The present experiments were designed to study what the increased sympathetic activity accompanying exercise might do to the blood circulation in the active muscles Rhythmic exercise of the forearm muscles was performed and lower body suction was used to increase the activity of the sympathetic adrenergic fibers to the resistance vessels in these muscles While rhythmic leg exercise would have provided the normal stimulus for the increased sympathetic activity lower body suction was chosen in preference The latter maneuver causes little alteration in systemic arterial blood pressure so that changes in forearm blood flow with and without suction could be compared directly as indices of changes in vascular resistance Reference has already been made to the difficulty of accurately measuring forearm blood flow by plethysmography when the exercise of the forearm muscles is severe The reduction in venous capacity probably by increased filtration means that on application of the venous collecting cuff the capacity system is filled in one and a half or two heart beats thus the slope of volume increase is difficult to interpret Leg exercise since it is accompanied by a sustained reflex constriction of the forearm veins (1) would have added to this problem whereas application of suction at most causes a minor and transient venoconstriction (32)

Thus lower body suction was used in the present study to increase the sympathetic vasoconstrictor activity during and after exercise The forearm exercise by itself caused some increase in sympathetic activity and more so during

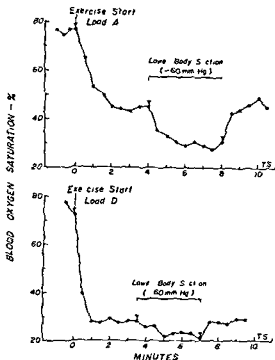


Fig 6 Effect of lower body suction on oxygen saturation in a deep forearm vein during continuous exercise of forearm muscles

sumption of the forearm during lower body suction, a constant arterial oxygen saturation of 96 %, and no change in the distribution of venous blood, the decrease in venous oxygen saturation with lower body suction corresponded to an average decrease in blood flow of 19 % at the lowest work load (range 11 to 27 %, 3 subjects) The correspon ing figure for the next work load wa 6 % (4 to 8 %, n = 2) and for th heaviest work load 9 % (4 to 16 % n = 3) For all experiments at the dif ferent loads taken together, the averag decrease was 14 % ($P < 0\,005$, n = 11) It was more marked at the lower wor loads, with a negative correlation coef ficient between the percentage decreas in flow and the work level (r = —0 62 $P < 0\,05$, n = 11) Considering th average 5 % decrease in perfusion pres sure with lower body suction, the aver age vascular resistance was not changed at the two highest work loads, but in creased by about 14 % at the lowest load

In the postexercise period after 2 1/2 min at load D, the initial increase in deep venous oxygen saturation at the end of the control exercise exceeded the resting value in two of three subjects, peak values being reached about 2 min after exercise The initial increase in oxygen saturation immediately after exercise was less markedly influenced by lower body suction than the saturation values subsequent to 1 min after exer

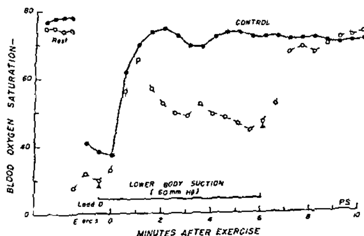


Fig 7 Effect of lower body suction on oxygen saturation in a deep forearm vein after exercise of the forearm muscles

during and after exercise caused by the sympathetic activation does not cause an oxygen debt. The decreased amount of blood delivered to the muscles is compensated for by increased oxygen extraction Ordinarily after local exercise of the forearm muscles, as demonstrated previously by Love (27), the oxygen saturation of the effluent blood from the forearm muscles increases to exceed the resting level, so that the arteriovenous oxygen difference is less than the resting value At this time, therefore, the muscles are oversupplied with oxygen, and this makes it unlikely that hypoxia plays any role in the increased flow after muscle contractions The present experiments demonstrate that if the sympathetic fibers are activated at this time, the venous oxygen saturation is prevented from rising above the resting value, it could be decreased by one third without delay of the metabolic recovery Thus the sympathetic stimulation has provided a fine adjustment of the local metabolic mechanisms that bring about increased blood flow to active muscles during exercise and thus a more economical ratio of blood flow to oxygen consumption is achieved

The mechanism of interaction between the sympathetic constrictor fibers and the local dilator mechanism is unknown Presumably the local metabolites by acting on the precapillary sphincters control the area of capillary bed which is perfused The volume of blood which perfuses this bed may be controlled both by metabolites and by the sympathetic nerves If the nerves act at the same site as the metabolites they could modify the degree of vasodilatation so that there was maximal extraction of oxygen from the blood passing through the capillaries The same effect would be produced if the metabolites and the sympathetic nerves acted at different sites on the resistance vessels The experiments of Renkin and Rosell (30) indicate that sympathetic adrenergic vasoconstrictor nerves act on both precapillary sphincters and upstream resistance vessels in resting skeletal muscle The effect of the sympathetic constrictor fibers on the precapillary sphincters seems, however, to be rapidly overcome by vasodilator metabolites when blood flow is decreased or metabolism increased (9) Hilton (18) found that the dilatation of the femoral artery in the cat after contraction of the muscles of the lower leg followed a similar time course to that of the postcontraction vasodilatation within the muscle Like the latter, the femoral dilatation was independent of any connection with the central nervous system In humans Crockford *et al* (13) found evidence for a spread of vasodilatation in the forearm skin possibly mediated by conduction in the smooth muscle of the arterial wall Thus with exercise a dilatation of precapillary sphincters caused by metabolites might be accompanied by a spread of the dilatation to upstream vessels the increased nervous activity might act at this latter site

The present observation that the metabolic recovery after exercise was not noticeably changed by the reflex reduction of blood flow during and after exercise is supported by observations of Dornhorst and Whelan (15) and Blair *et al* (4) These authors found that a

the heavy loads, as evidenced by the more marked increases in heart rate and arterial blood pressure The lower body suction, however, had a more pronounced effect on heart rate and was probably the more potent stimulus to the sympathetic nervous system, in its vasoconstrictor effect on resting muscle is was comparable to that caused by severe (820 kpm/min) leg exercise

At rest, both skin and muscle vessels are affected by the increased sympathetic activity during lower body suction (6, 14) Further evidence that the muscle vessels are constricted is provided by the present experiments since the clearance of xenon-133 depots from muscle always was decreased by suction when the limb was at rest Also, in the resting forearm, application of suction reduced the blood flow in the deep vein draining the muscles, so that the fixed withdrawal rate of blood at 2 5 ml/min could not be maintained

When forearm blood flow at the site of maximal muscle mass was measured by strain gauge plethysmography it was shown to be decreased between rhythmic contractions of the forearm muscles by sympathetic stimulation when the exercise was mild As the exercise increased in severity, the relative decrease in flow, compared with control exercise without the augmented sympathetic activity was less, with maximal or near maximal activity as noted also in dogs (29) and cats (24), no effect of significance was noted

The plethysmographic findings are reinforced by the xenon 133 clearance studies which give integrated values for muscle blood flow during and between contractions, the blood flow in small segments of the flexor muscles was reduced by the increased sympathetic adrenergic activity when the exercise was mild, and this effect was gradually abolished as the degree of muscle activity increased Thus the blood flow through the resistance vessels in muscle, whose caliber is regulated by metabolic changes in the active muscles, can if the concentration of metabolites is not excessive, be influenced by changes in sympathetic activity in adrenergic nerves This conclusion is reinforced by the studies of forearm blood flow following the periods of rhythmic exercise Immediately after the most severe exercise when the local vasodilator metabolites in muscle are at their maximum, and the resistance vessels as a consequence are widely dilated, lower body suction had little effect in reducing the blood flow However, in less than a minute as the concentration of metabolites and the blood flow began to decrease, the increased sympathetic nervous activity caused the blood flow to decline very rapidly, so that the amount of blood flowing through the forearm after exercise in excess of the resting level was greatly reduced This substantiates the findings of Fewings et al (16) who showed that tilting recumbent subjects into the feet down position reduced the magnitude and duration of postexercise hyperemia following 1, 2, or 3 min periods of forearm exercise because of a sympathetic vasoconstrictor reflex The reduction in the peak blood flow however was significant for the 3 min period of exercise only

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This reduction in blood flow both

When exercise was mild, the muscle blood flow was reduced by the increased adrenergic activity, this effect lessened as the exercise increased in severity, so that with maximal activity no effect of significance was observed The decrease in flow during and after exercise caused by the increased sympathetic action was compensated for by greater oxygen extraction, so that no debt occurred and the metabolic recovery was not prolonged It is postulated that during normal exercise the sympathetic adrenergic nerves modulate the local dilator mechanism in active muscles to maintain the most economical ratio of blood flow to oxygen consumption

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mechanical reduction in forearm blood flow after exercise did not lead to any prolongation of the postexercise hyperemia It would appear that the rate of recovery of the blood flow to the resting level after exercise is time dependent rather than flow dependent Dornhorst and Whelan (15) suggested that the postexercise hyperemia may be caused by a metabolite whose transport from the cells to the blood is limited by diffusion rather than blood flow when the blood flow exceeds a certain minimal value, or the metabolite oxidizes at a rate dependent on its concentration and independent of local oxygen tension when this exceeds some low figure

The relation of these experimental findings to exercise under normal conditions may now be examined Supine exercise causes increased adrenergic nerve activity proportional to the severity of the exercise Tilting head up, which is equivalent in its hemodynamic effects to lower body suction (6), does the same With upright exercise, the increased sympathetic activity from the postural reflexes may reinforce that caused by the exercise, this would explain the finding that during upright exercise the cardiac output is about 2 liters/min less than during supine exercise for the same oxygen consumption (3)

The implication of the present findings is that the increased adrenergic nerve activity not only increases resistance to flow in vascular beds outside the active muscles but also acts to adjust the metabolic vasodilatation throughout the total active musculature, so that oxygen extraction from the blood is optimal There is no inhibition of flow to the most active muscles, where oxygen supplies are completely flow dependent, but flow is reduced to less active muscles, where the local metabolic changes result in a greater flow than necessary for oxygen demands It is the performance of the cardiovascular system that dictates maximal exercise tolerance Skeletal muscles constitute about 40 % of the total body mass, and much of this may be active during generalized exercise in the upright position The sympathetic system, by acting as the fine adjustment of metabolically increased flow, not only reduces the load on the left ventricle during exercise but also, by reducing the decrease in total systemic vascular resistance, helps to maintain the systemic arterial blood pressure and hence the perfusion pressure Thus the most active parts are assured of the maximal possible blood flow

Summary

The interaction has been studied between increased adrenergic nerve activity and the metabolic dilatation of the resistance vessels in active human forearm muscles Forearm blood flow was measured by venous occlusion plethysmography and muscle blood flow by xenon 133 clearance during and after rhythmic exercise of the forearm muscles The oxygen saturation of blood draining the muscles was determined The activity of the sympathetic vasoconstrictor fibers to the forearm was augmented by application of subatmospheric presssure to the lower part of the body

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From the Department of Clinical Physiology, Allmanna Sjukhuset, Malmo, Sweden

Zonal Distribution of Pulmonary Blood Flow during Unilateral Hypoxia

A broncho-radiospirometric study

By

MÅNS ARBORELIUS JR, SVEN-ERIC LINDELL and ROBERT O MALMBORG

Unilateral hypoxia constricts the blood vessels in the hypoxic lung in man (1, 6, 8) This applies even if the oxygen concentration in the gas inhaled by the hypoxic lung is as high as 15 per cent (2) Little is known about the mechanisms involved However, Fowler and Read (5) studying the cardiogenic oscillations of expired gas tensions with a mass-spectrometer, concluded that the hypoxic constriction of the blood vessels occurred mainly in the lower (basal) zones of the lungs We have used radioactive xenon and external detectors which makes possible a more direct study of the zonal distribution of the hypoxic vasoconstriction

Material

The study was carried out in three young male subjects with normal spirometries and without any demonstrable lung X-ray changes In addition, they had no history of pulmonary disease

Methods

The bronchospirometric technique was the same as described by Arborelius (2) The air ways were anesthetized with 4 ml of Citanest® without epinephrine Carlens' catheter number 39 was used The distribution of the pulmonary blood flow was measured from the distribution of radioactivity in the lungs after an intravenous injection of ^{133}Xenon dissolved in saline This technique was first described by Ball et al (3) and Dollery et al (4) We have used this technique as modified by Miorner (7) for improved precision in repeated measurements The subjects were examined in the *supine* position The detectors were arranged as shown in Fig 1 from Miorner (7) The eight scintillation detectors were coupled in pairs One detector in each pair was placed in front of the subject and the other behind The impulses from the two detectors in each pair were added and recorded on one channel in a four channel

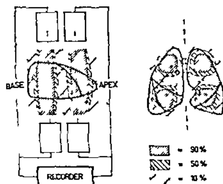


Fig 1 The arrangement of detectors collimators and recorder for measuring regional pulmonary blood flow with ^{133}Xenon (7)
The activity recorded with the xenon source in the central axis between the crystals was taken as 100 per cent

taperecorder Each pair of detectors saw a quadrant of the lung from the hilus towards the apex or the base For measurement of the distribution of pulmonary perfusion 1 mC of ^{133}Xenon dissolved in saline was injected via a catheter in the superior caval vein during breath holding after a normal expiration The level (amplitude) of radioactivity recorded from each field was considered directly proportional to the blood flow in that field The sum of the amplitudes of the four channels was considered to equal total pulmonary perfusion The blood flow through each field was expressed in per cent of this total blood flow The blood flow through each lung was calculated as the sum of the blood flow through the apex and the base.

The arterial blood oxygen and carbon dioxide tensions were measured directly with conventional electrode techniques

After the Carlens tube had been introduced the subject was placed in the supine position between the detectors and connected to low resistance valves for the supply of breathing gases The first injection of xenon was made when he had been breathing air bilaterally for five minutes after the intubation At the same time arterial blood was sampled for blood gas analyses The right lung then inhaled nitrogen and the left lung oxygen for ten minutes Further xenon injections were made at the third and tenth minute of this period A final measurement was obtained after another five minutes of air breathing

Results

The results are presented in Table I The right lung s part of the total pulmonary perfusion in the control period, i e during bilateral air breathing was between 49 and 53 per cent which is within the normal range (7) The relative blood flow in the right apical field and the left apical field was close to unity in all three subjects during the control period The same applied to the blood flows in the basal parts of the lungs However as may be seen in Table I the ratio between the blood flow through the apical part and the basal part (apex/base ratio) in the same lung ranged from 0 45 to 1 12 in the control period This wide variation in apex to-base ratio is also found among the normal subjects (7) It is probably due to different shapes of the lungs in different individuals and to difficulties in positioning the detectors exactly in the same way in different individuals However, the detectors saw the same quantity of lung throughout the whole investigation and, as

TABLE I Redistribution of pulmonary blood flow during unilateral hypoxia Apical and basal blood flows are given in per cent of *total pulmonary blood flow* (R = ringt lung, L = left lung, A = apex, B = base)

		Control		3 min		10 min		5 min	
		R Air	L Air	R N_2	L O_2	R N_2	L O_2	R Air	L Air
H E	A	25 5	24 6	16 9	37 3	13 2	40 2	25 4	23 4
♂	B	28 0	21 9	13 4	32 4	10 1	36 5	25 5	25 7
20 y	A + B	53 5	46 5	30 3	69 7	23 3	76 7	50 9	49 1
	A/B	0 91	1 12	1 26	1 15	1 31	1 10	1 00	0 91
C C	A	15 6	15 8	11 1	19 9	10 4	23 2	16 8	14 5
♂	B	33 1	35 5	21 3	47 7	16 7	49 7	34 1	34 6
19 y	A + B	48 7	51 3	32 4	67 6	27 1	72 9	50 9	49 1
	A/B	0 47	0 45	0 52	0 42	0 63	0 47	0 49	0 42
Ch N	A	24 3	25 1	17 5	32 8	16 2	35 5	25 9	27 8
♂	B	25 0	25 6	16 9	32 8	13 5	34 8	21 3	25 0
23 y	A + B	49 3	50 7	34 4	65 6	29 7	70 3	47 2	52 8
	A/B	0 97	0 98	1 03	1 00	1 20	1 02	1 22	1 11

the subjects could not move, changes in the apex to-base ratio were secondary to changes in the relative blood flow In *all three* subjects nitrogen-breathing with the right lung was associated with a pronounced reduction in the blood flow *to that lung* In all the subjects this remarkable redistribution of pulmonary *blood flow* had taken place already after three minutes of unilateral nitrogen breathing It may be calculated that the blood flow of the hypoxic lung decreased *to about half* of its original volume This redistribution of pulmonary blood flow during pronounced unilateral hypoxia agrees closely with that found by Arborelius (2) although the present technique of measurement is different It is obvious that the vasoconstriction responsible for this redistribution of the blood flow occurred both in the apical and the basal parts of the hypoxic lung

A comparison between the apex *to* base ratio for pulmonary blood flow in each individual subject during the control period and during the test periods reveals that this ratio regularly increased in the hypoxic lung, whereas it was unchanged or decreased in the control lung breathing oxygen This indicates a stronger reaction to the hypoxic stimulus in the blood vessels of the basal parts of the lungs The changes in the apex to-base ratio are greater in these experiments than those found at duplicate measurements in normal man (7) The control measurements carried out at the end of the experiment after five minutes of air breathing showed that distribution of pulmonary blood flow was now almost the same as during the control period before hypoxia in two of the subjects In the third subject (Ch N) the change in the apex to-base ratio did

TABLE II Total ventilation arterial blood gases and pH during bilateral air breathing and after 10 minutes of unilateral hypoxia

Subject		$\dot{V}_E$ l/min	$P_{a_{O_2}}$ mm Hg	$P_{a_{CO_2}}$ mm Hg	pH_a
H E.	air air	7 0	67	43	7 37
	N_2—O_2	7 7	126	45	7 37
C C.	air air	5 1	78	42	7 36
	N_2—O_2	7 2	84	43	7 36
Ch N	air air	6 8	80	44	7 39
	N_2—O_2	7 2	78	45	7 39

not occur within the first three minutes of hypoxia and the return to control level was not accomplished within five minutes of air breathing The analysis of arterial blood gases showed that there was no decrease in arterial oxygen tension although the right lung was breathing pure nitrogen (Table II) Although the total ventilation increased by about 10 per cent the carbon dioxide tension rose by about 1 mm This indicates uneven ventilation perfusion within the lungs

Discussion

By external measurements of the radioactivity in the lungs after intravenous injections of radioactive xenon we have been able to confirm previous observations that hypoxia constricts the pulmonary blood vessels in man The hypoxic vasoconstriction occurred within three minutes and was most pronounced in the basal parts of the lungs Thus our results give further support to the conclusion by Fowler and Read (5) that there is a preferential lower zone vasoconstriction in response to hypoxia Fowler and Read (5) using a more indirect method of studying the distribution of pulmonary blood flow thought that lower zone vasoconstriction was a more likely response to alveolar hypoxia than general vasoconstriction However, our subjects showed quite a pronounced reaction to hypoxia also in the apical parts of the lung Thus we disagree somewhat with Fowler and Read as regards the participation of the apical blood vessels in the hypoxic vasoconstriction, but we still think that their original observation of the preferential lower zone vasoconstriction during hypoxia is very interesting It is tempting to speculate as do Fowler and Read that this represents an evolutionary response to man s adoption of the erect posture It is also possible that this stronger reaction to hypoxia contributes to the decrease in the perfusion of the basal parts of the lungs in mitral valvular disease

Summary

Radiospirometry with intravenously injected [133]Xenon was used to measure the distribution of pulmonary blood flow during bronchospirometry in three healthy volunteers

TABLE I Redistribution of pulmonary blood flow during unilateral hypoxia Apical and basal blood flows are given in per cent of total pulmonary blood flow (R = right lung, L = left lung, A = apex B = base)

		Control		3 min		10 min		5 min	
		R Air	L Air	R N_2	L O_2	R N_2	L O_2	R Air	L Air
H E	A	25.5	24.6	16.9	37.3	13.2	40.2	25.4	23.4
♂	B	28.0	21.9	13.4	32.4	10.1	36.5	25.5	25.7
20 y	A + B	53.5	46.5	30.3	69.7	23.3	76.7	50.9	49.1
	A/B	0.91	1.12	1.26	1.15	1.31	1.10	1.00	0.91
C C	A	15.6	15.8	11.1	19.9	10.4	23.2	16.8	14.5
♂	B	33.1	35.5	21.3	47.7	16.7	49.7	34.1	34.6
19 y	A + B	48.7	51.3	32.4	67.6	27.1	72.9	50.9	49.1
	A/B	0.47	0.45	0.52	0.42	0.63	0.47	0.49	0.42
Ch N	A	24.3	25.1	17.5	32.8	16.2	35.5	25.9	27.8
♂	B	25.0	25.6	16.9	32.8	13.5	34.8	21.3	25.0
23 y	A + B	49.3	50.7	34.4	65.6	29.7	70.3	47.2	52.8
	A/B	0.97	0.98	1.03	1.00	1.20	1.02	1.22	1.11

the subjects could not move, changes in the apex to-base ratio were secondary to changes in the relative blood flow In all three subjects nitrogen breathing with the right lung was associated with a pronounced reduction in the blood flow to that lung In all the subjects this remarkable redistribution of pulmonary blood flow had taken place already after three minutes of unilateral nitrogen breathing It may be calculated that the blood flow of the hypoxic lung decreased to about half of its original volume This redistribution of pulmonary blood flow during pronounced unilateral hypoxia agrees closely with that found by Arborelius (2) although the present technique of measurement is different It is obvious that the vasoconstriction responsible for this redistribution of the blood flow occurred both in the apical and the basal parts of the hypoxic lung

A comparison between the apex to-base ratio for pulmonary blood flow in each individual subject during the control period and during the test periods reveals that this ratio regularly increased in the hypoxic lung whereas it was unchanged or decreased in the control lung breathing oxygen This indicates a stronger reaction to the hypoxic stimulus in the blood vessels of the basal parts of the lungs The changes in the apex to base ratio are greater in these experiments than those found at duplicate measurements in normal man (7) The control measurements carried out at the end of the experiment after five minutes of air breathing showed that distribution of pulmonary blood flow was now almost the same as during the control period before hypoxia in two of the subjects In the third subject (Ch N) the change in the apex to-base ratio did

Department of Clinical Physiology and Department of Surgery I University of Goteborg Sweden

The General Hemodynamics and Pulmonary Gas Exchange at Various Distributions of the Blood Volume in Man

By

J Bjure, A Carlsten, U Ljungqvist and N J Nilsson

When the position of the body is changed from lying to standing there is a re distribution of blood from the heart and lungs to the peripheral circulation in the lower part of the body for references see Sjostrand (27) This hydrostatic shift of blood to the legs has consequences for the central circulation in normal sub jects The filling of the heart is decreased and there is a conspicuous fall in the stroke volume combined with an in creased heart rate These hemodynamic effects are still more striking in patients with varicose veins (1 19) but can be reduced in these patients when bandages are applied to the legs The same effect of bandages on the hemodynamics can be demonstrated during sitting exercise in patients with varicose veins (13) in dicating that also at work central cir culation is influenced by pooling of blood in the varicose veins of the legs

From the investigation by Bates and Pierce (3 we know that the pulmonary diffusing capacity (D_L) falls when the body posture is changed from supine to erect Inflation of an antigravity pressure suit over the lower part of the body increases the breath holding dif fusing capacity (16, 23) as well as the steady state diffusing capacity (22), presumably due to increase in the pul monary capillary blood volume (25)

When duplicate determinations of D_L with the steady state technique were made at rest in the supine position in normal subjects and patients with only moderately advanced pulmonary disease (5) D_L was found to fall from the first to the second determination With the assumption that changes in D_L reflect variations in pulmonary capillary blood volume the hypothesis was launched that the volume of blood centrally was smaller during the second determination due to redistribution of blood to the peripheral circulation

The present study was undertaken in order to investigate further the inter relationship between the intrathoracic blood volume gas exchange and general hemodynamics at rest and during physi cal exercise Thus measurements were made on normal subjects *at rest supine* with an antigravity pressure suit over the lower half of the body and during

In the supine position unilateral hypoxia (100 % N_2) decreased the relative blood flow through the hypoxic lung to about half the original value The decrease was more pronounced in the basal part of the lung, indicating greater vasomotricity in this region No redistribution between the apex and the base occurred in the oxygen breathing control lung

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Table II Anthropometric and hemodynamic data during exercise (Procedure B)

		Sex	Age	Height cm	Weight kg	Brachial artery pressure mm Hg			HR	Q	SV
						S	D	M			
1	a	F	40	167	74	184	95	135	125	14 5	116
	b					163	83	111	131	12 8	98
2	a	M	47	182	84	173	86	118	108	12 4	115
	b					168	89	113	123	10 1	82
3	a	F	50	165	79	205	99	123	128	13 6	106
	b					211	95	136	155	12 1	78
4	a	F	40	173	64	165	82	120	115	11 1	97
	b					179	79	115	130	9 8	75
5	a	F	53	168	76	229	121	162	133	12 7	95
	b					219	112	160	140	11 2	80
6	a	F	42	170	82	144	72	97	118	10 3	87
	b					150	78	100	131	9 4	71
Mean values											
	a					183	93	126	121	12 4	103
	b					182	89	123	135	10 9	81
Statistical significance						—	—	—	+	+	+

a) Period with bandages on the legs
b) Period without bandages on the legs
Statistical significance see Table I
The symbols are the same as in Table I

These studies aimed at large changes in the central blood volume in the same person They were made in the supine position a) during inflation to 100 mm Hg of an antigravity pressure suit to force blood centrally from the lower part of the body and b) during venous occlusion of the lower limbs to withdraw blood from the lungs and heart Two of the subjects were studied twice with about three weeks interval Four of the eight studies started with the pressure suit and the remaining four with the cuffs around the thighs The suit was not placed higher up than just below the costal margin since Ross et al (23) have found that with the suit placed up to the nipples the vital capacity decreases and the expected rise in diffusing capacity with inflation does not occur

Procedure B One male and five female patients with moderate varicose veins on both legs were studied One patient (no 6) had been treated with injections in one leg ten years prior to the study Spirometry revealed no pathological signs and the physical performances on a bicycle ergometer were considered fairly normal Anthropometric data on the patients are given in Table II

As the first step in procedure B the legs of the patients were bandaged up to the groins after the patients had been lying with elevated legs for about 10

TABLE I Anthropometric and hemodynamic data at rest (Procedure A)

		Sex	Age	Height cm	Weight kg	Brachial artery pressure mm Hg			HR	Q	SV
						S	D	M			
1	b	M	25	179	73	144	81	105	60	5.2	87
	a					157	81	116	58	5.8	100
2	b	M	32	169	66	143	84	109	76	4.5	59
	a					152	93	117	68	4.9	72
3	a	M	22	196	82	160	88	113	66	8.1	123
	b					136	72	88	64	6.6	103
	b					127	63	81	58	5.5	95
	a					128	95	99	58	6.8	117
4	b	M	22	184	73	133	81	102	72	5.7	79
	a					129	77	96	86	6.5	76
	a					137	76	96	76	5.6	74
	b					126	76	88	88	5.3	60
5	a	M	30	185	76	136	82	95	74	7.4	100
	b					120	71	90	76	7.2	95
6	a	M	29	180	70	128	80	99	52	4.9	94
	b					130	83	94	56	5.8	104
Mean values	a					141	84	104	67	6.3	95
	b					132	76	95	69	5.7	85
Statistical significance						—	—	+	—	+	+

a) Period during inflation of antigravity pressure suit
b) Period during venous occlusion with cuffs on the thighs
Statistical significance + indicates a probability $(P) < 0.05$ that the mean of the differences (a—b) is caused by random factors — indicates $P > 0.05$

S	systolic	HR	heart rate — beats/min
D	diastolic	Q	cardiac output — l/min
M	mean	SV	stroke volume — ml

pooling of blood in the legs by venous occlusion, and on patients with varicose veins *during sitting exercise* with and without bandages on the legs

Material and procedure

Procedure A The experiments with the antigravity suit were made on six healthy male volunteers They were blood donors with various occupations, 22—32 years old No experiments were performed until at least one month after a venesection Some days before the study proper the volunteers were examined and a work test and a determination of static and dynamic lung volumes were made They all had normal physical performance and spirometry Special emphasis was placed on the history regarding the existence of heart and lung diseases Anthropometric data on the subjects are given in Table I

TABLE II Anthropometric and hemodynamic data during exercise (Procedure B)

		Sex	Age	Height cm	Weight kg	Brachial artery pressure mm Hg S	D	M	HR	Q	SV
1	a	F	40	167	74	184	95	135	125	14.5	116
	b					163	83	111	131	12.8	98
2	a	M	47	182	84	173	86	118	108	12.4	115
	b					168	89	113	123	10.1	82
3	a	F	50	165	79	205	99	123	128	13.6	106
	b					211	95	136	155	12.1	78
4	a	F	40	173	64	165	82	120	115	11.1	97
	b					179	79	115	130	9.8	75
5	a	F	53	168	76	229	121	162	133	12.7	95
	b					219	112	160	140	11.2	80
6	a	F	42	170	82	144	72	97	118	10.3	87
	b					150	78	100	131	9.4	71
Mean values											
	a					*183*	*93*	*126*	*121*	*12.4*	*103*
	b					182	89	123	135	10.9	81
Statistical significance						—	—	—	+	+	+

a) Period with bandages on the legs
b) Period without bandages on the legs
Statistical significance see Table I
The symbols are the same as in *Table I*

These studies aimed at large changes in the central blood volume in the same person They were made in the supine position a) during inflation to 100 mm Hg of an antigravity pressure suit to force blood centrally from the lower part of the body and b) during venous occlusion of the lower limbs to withdraw blood from the lungs and heart Two of the subjects were studied twice with about three weeks interval Four of the eight studies started with the pressure suit and the remaining four with the cuffs around the thighs The suit was not placed higher up than just below the costal margin since Ross et al (23) have found that with the suit placed up to the nipples the vital capacity decreases and the expected rise in diffusing capacity with inflation does not occur

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	b					136	72	88	64	6 6	103
	b					127	63	81	58	5 5	95
	a					128	95	99	58	6 8	117
4	b	M	22	184	73	133	81	102	72	5 7	79
	a					129	77	96	86	6 5	76
	a					137	76	96	76	5 6	74
	b					126	76	88	88	5 3	60
5	a	M	30	185	76	136	82	95	74	7 4	100
	b					120	71	90	76	7 2	95
6	a	M	29	180	70	128	80	99	52	4 9	94
	b					130	83	94	56	5 8	104
Mean values	a					141	84	104	67	6 3	95
	b					132	76	95	69	5 7	85
Statistical significance						—	—	+	—	+	+

a) Period during inflation of antigravity pressure suit
b) Period during venous occlusion with cuffs on the thighs
Statistical significance + indicates a probability (P) < 0 05 that the mean of the differences (a—b) is caused by random factors — indicates P > 0 05

S	systolic	HR	heart rate beats/min
D	diastolic	Q	cardiac output — l min
M	mean	SV	stroke volume — ml

pooling of blood in the legs by venous occlusion, and on patients with varicose veins *during sitting exercise* with and without bandages on the legs

Material and procedure

Procedure A The experiments with the antigravity suit were made on six healthy male volunteers They were blood donors with various occupations, 22—32 years old No experiments were performed until at least one month after a venesection Some days before the study proper, the volunteers were examined and a work test and a determination of static and dynamic lung volumes were made They all had normal physical performance and spirometry Special emphasis was placed on the history regarding the existence of heart and lung diseases Anthropometric data on the subjects are given in Table I

Table III Respiratory data at rest (Procedure A)

		$\dot{V}_E$	f	$\dot{V}_{O_2}$	RQ	$\dot{V}_A$	$P_{a_{CO_2}}$	pH	P_{a_O}	$P_{A_O}-P_{a_O}$	$D_{L_{CO}}$
1	b	10 2	5	220	0 84	4 2	39	7 38	103	2	20 6
	a	5 4	5	159	0 75	2 6	40	7 38	92	5	26 2
2	b	5 9	12	196	0 85	4 2	35	7 42	108	1	21 6
	a	6 3	12	207	0 77	3 7	38	7 38	105	0	29 7
3	a	16 8	22	248	0 99	7 4	29	7 47	114	7	28 7
	b	8 3	16	204	0 96	6 1	28	7 48	116	5	25 2
	b	6 7	14	190	0 79	4 5	29	7 46	117	0	34 7
	a	7 5	18	219	0 74	3 8	38	7 39	111	0	36 9
4	b	8 6	16	251	0 90	5 8	34	7 41	106	3	35 1
	a	6 5	16	253	0 80	4 3	41	7 38	106	0	46 7
	a	6 1	12	251	0 77	4 3	39	7 41	100	4	33 8
	b	7 2	15	247	0 79	4 5	38	7 40	100	5	30 5
5	a	10 3	16	288	0 81	5 3	39	7 41	104	0	24 8
	b	18 6	17	257	0 77	4 3	40	7 39	93	5	31.5
6	a	7 7	19	200	0 84	3 9	38	7 35	104	3	32 2
	b	9 8	14	231	0 86	4 4	39	7 34	99	6	29 8
Mean values											
	a	8 3	15	228	0 81	4 4	38	7 40	105	2	32 4
	b	9 4	14	225	0 85	4 8	35	7 41	105	3	28 6
Stat sign		—	—	—	—	—	—	—	—	—	+

a) Period during inflation of antigravity pressure suit
b) Period during venous occlusion with cuffs on the thighs
Statistical significance see Table I

$\dot{V}_E$	total ventilation — l/min	P_{a_O}	arterial oxygen tension — mm Hg
f	respiratory frequency — breaths/min	$P_{A_O}-P_{a_O}$	alveolo-arterial oxygen tension difference — mm Hg
$\dot{V}_O$	oxygen consumption — ml/min	$D_{L_{CO}}$	pulmonary diffusing capacity for carbon monoxide — ml/(mm Hg min)
RQ	*respiratory quotient*		
$\dot{V}_A$	alveolar ventilation — l/min		
P_{CO}	arterial carbon dioxide tension — mm Hg		

In conclusion significant and almost parallel changes were found in pulmonary diffusing capacity cardiac output and stroke volume without significant changes *in total ventilation tidal volume* or alveolar ventilation

Procedure B Studies during exercise in the patients with bilateral varicose veins showed more dramatic effects on general hemodynamics and pulmonary gas exchange Although no changes could be found in brachial artery pressures, there were marked differences in heart rate cardiac output and stroke volume Thus during the last part of the exercise when the bandages were taken away the heart rate rose on an average 14 beats per minute or 12 per cent, the mean cardiac

min Thereafter an exercise in the sitting posture was started on a bicycle ergometer The work load for the female subjects was 300 kpm/min, and for the male 450 kpm/min Data on the general hemodynamics and pulmonary gas exchange were collected during the exercise Two studies were made, one before and one after the bandages had been removed without interruption of the exercise Thus, for practical reasons, the period without bandages always followed the period with bandages

Methods

All subjects arrived at the laboratory in the morning after a light meal They were allowed to rest supine for about 30 minutes before polyethylene catheters were inserted into one or two arteries and a vein in the elbow region

Cardiac output was determined in *procedure A* by a single determination with an indicator dilution technique using I^{131}-Hippuran as an indicator Arterial samples were collected with a fraction collector In *procedure B*, the cardiac output was determined by at least duplicate estimations with the dye dilution technique, using a specially developed cuvette densitometer in connection with an Atlas oximeter (20, 21) and with Cardio green as the indicator The withdrawn blood was reinfused after the recording of one or occasionally two dye curves The subject's blood volume was thus kept constant The methodological details are described by Grimby and Nilsson (12)

Gas exchange Expired air was collected in Douglas bags and analyzed by the Scholander technique The arterial oxygen tension was measured with the method of Gleichmann and Lubbers (11), and arterial carbon dioxide tension according to Berglund, Malmberg and Stenhagen (4) The pulmonary diffusing capacity was estimated using the steady state CO technique described by Filley, McIntosh and Wright (10), modified according to Linderholm (18) to correct for the back pressure of carbon monoxide in the pulmonary capillaries The technique is described in detail by Bjure (5)

Statistical methods The differences between the values for the different experimental situations were tested with Students t test A 5 % significance level ($P < 0\,05$) was used throughout

Results

Procedure A Inflation of an antigravity pressure suit forces blood from the periphery to the central circulation, whereas the venous occlusion with cuffs round the thighs is accompanied by a redistribution of central blood to the periphery The redistribution of the volume of the blood occurring when the suit is deflated and blood is pooled distal to the cuffs gave the expected significant fall in the mean brachial artery pressure Cardiac output also fell, on an average 0 6 liter/min, almost entirely due to the fall in stroke volume which averaged 10 ml or 11 per cent (Table I)

There was a significant decrease in D_L, averaging 3 8 ml/(min mm Hg) or 12 per cent, without significant changes in total ventilation or tidal volume (Table III)

suit applied To achieve larger changes in the distribution of blood in the body we have compared the results during *inflation* of the pressure suit with those during venous occlusion by cuffs around the thighs in normal subjects

With our experimental procedure there is a shift of blood from the central circulation to the periphery as judged from the significant decrease in stroke volume, cardiac output and mean brachial artery pressure This would imply also a fall in pulmonary blood volume This volume reduction might then be responsible for the decrease in the diffusing capacity of the lung Johnson Spicer, Bishop and Forster (15) suggested that the stroke volume would be approximately equal to the volume *of the pulmonary capillary bed* an opinion which seems to be supported by the finding by Bjure (5) of a significant *correlation between stroke volume and* pulmonary diffusing capacity during exercise Also during the infusion of histamine an increase in pulmonary diffusing capacity has been demonstrated roughly related to the rise in stroke volume (7) The results of the present study in procedure A fit well with this concept since diffusing capacity varied almost in parallel with stroke volume The finding of a rise in diffusing capacity with inflation of a pressure suit is in accordance with the results by Ross et al (23) who used the single breath technique with the subjects in the sitting position With the steady state technique there is only one earlier study (22) showing that on three subjects the diffusing capacity was higher with pressure suit than without. These authors found so large an increase in ventilation, that it could be expected to influence the diffusing capacity

The redistribution of blood to the periphery in procedure A resulted in a significant fall of 11—12 per cent in stroke volume and diffusing capacity In circumstances inferring a fall in pulmonary blood volume the upper lung regions are especially depleted of blood in comparison with the lower regions From studies by Severinghaus and Stupfel (26) and Barr (2) we know that under such conditions the alveolar ventilation increases with a consequent fall in arterial CO_2 tension In the present study these changes were not statistically significant

Varicose veins have marked effects on *the central circulation both at rest* (1, 23) and during exercise (13) These effects can be reduced by bandaging the legs The *earlier study during exercise* (13) was performed with and without bandages on the same patient with an interval of 2 hours In the present study we measured the general hemodynamics and pulmonary gas exchange with and without bandages without interrupting the exercise From the hemodynamic results it can be concluded that a shift of blood to the periphery occurred which was even more marked than during inflation of the pressure suit and venous pooling of blood in the legs of the normals in the supine *position* This is judged from the 21 per cent fall in stroke volume and 12 per cent fall in cardiac output *Since the studies were* made during exercise in the sitting *position* hydrostatic forces have *more* influence on the distribution of blood

Table IV Respiratory data during exercise (Procedure B)

		$\dot{V}_E$	f	$\dot{V}_{O_2}$	RQ	$\dot{V}_A$	P_{aCO_2}	pH	P_{aO_2}	$P_{AO_2}-P_{aO_2}$	D_{LCO}
1	a	22 8	20	860	0 92	16 3	42	7 35	105	4	37 9
	b	22 8	22	839	0 89	19 2	34	7 36	92	21	29 0
2	a	30 8	18	1,382	0 83	24 9	40	7 42	82	21	36 1
	b	32 3	22	1,238	0 89	25 9	37	7 42	81	24	38 9
3	a	27 1	22	1,188	0 86	22 8	39	7 44	92	17	32 6
	b	33 6	26	1,212	0 94	30 8	32	7 45	95	20	28 5
4	a	31 9	11	1,005	0 89	25 0	31	7 42	100	14	22 3
	b	31 4	16	978	0 85	25 6	28	7 43	83	36	20 0
5	a	23 2	18	1 023	0 88	18 5	42	7 38	96	3	40 4
	b	23 1	22	1,030	0 82	18 8	39	7 40	85	15	37 9
6	a	27 1	24	929	0 92	23 2	32	7 43	78	34	29 8
	b	29 4	32	925	0 86	24 8	28	7 45	85	29	32 1
Mean values											
	a	27 2	19	1,065	0 88	21 8	38	7 41	92	16	33 2
	b	28 9	23	1 037	0 88	24 2	33	7 42	87	24	31 1
Stat sign		—	+	—	—	+	+	+	—	—	—

a) Period with bandages on the legs
b) Period without bandages on the legs
Statistical significance, see Table I

The symbols are the same as in Table III

output fell 1 5 l/min or 12 per cent, and there was an average fall in stroke volume of 22 ml or 21 per cent All these changes were statistically significant (Table II)

The respiratory data are given in Table IV On removing the bandages the alveolar ventilation increased significantly, the average rise being 2 4 liters per min or 11 per cent which is also shown in the mean decrease in arterial CO_2 tension of 5 mm Hg or 13 per cent The alveolo-arterial oxygen tension difference rose in all but one case (no 6) on an average by 8 mm Hg, this difference, however, was not significant The pulmonary diffusing capacity was not significantly changed In conclusion, with significant fall in cardiac output and stroke volume and rise in alveolar ventilation there was no change in pulmonary diffusing capacity

Discussion

Inflation of an antigravity pressure suit over the lower part of the body produces pulmonary vascular engorgement (8, 17, 22, 24) and acute pulmonary hypertension (9, 17) According to Ross et al (22) and Daly et al (9) this is achieved without significant increase in cardiac output

These studies on hemodynamics and pulmonary gas exchange have compared the results with and without the pressure

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within the lungs, with depletion at the top and congestion at the bottom This would be expected to lead to a rise in alveolar ventilation with a fall in arterial CO_2 tension and an increased alveolo-arterial oxygen tension difference Interestingly enough, changes of this type have been found in cases of recurrent pulmonary embolism (6, 28) The same findings were noted by Barr (2) during prolonged gravitational stress, he also recorded a fall in arterial oxygen saturation which he interpreted as the result of a low ventilation-perfusion ratio in the dependent parts of the lungs The results in the present study are of this nature, but with a decrease in diffusing capacity which is not significant It may be, however, that the rise in diffusing capacity, which is known to take place at the transition from rest to exercise (14), had not yet reached its maximum when our first determination was made, and that its persisting rise has counteracted the reduction after removal of the bandages

Summary

1 Six normal subjects were studied in the supine position both during pooling blood in the lower limbs and during inflation of an antigravity pressure suit (Procedure A)

2 Six patients with moderate bilateral varicose veins were investigated during sitting exercise with and without elastic bandages on the legs (Procedure B)

3 At both procedures the cardiac output and pulmonary diffusing capacity were determined

4 In procedure A we recorded during pooling significantly lower values of cardiac output and pulmonary diffusion capacity without significant changes in other respiratory data

5 In procedure B similar hemodynamic observations were obtained but the concomitant change in the pulmonary diffusing capacity was not significant. The alveolar ventilation was, however, significantly increased in this procedure.

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From MMLC (Military Medical Examination Center) and the Department of Clinical Physiology Karolinska sjukhuset, Stockholm and the Department of Clinical Physiology University of Goteborg Sweden

Capacity for Brief Exercise in Relation to Capacity for Prolonged Exercise in Man

By

Bjorn Ahlborg, Gunvor Ahlborg, Mattias Aurell and Klas Linroth

The ability of the circulation to meet the demand of physical work which requires the use of large muscle groups constitutes a measure of the physical working capacity In man this can be carried out in a number of ways The most common method for determining physical working capacity in Swedish hospitals is to determine the amount of work a subject can perform on a bicycle ergometer at a heart rate of 170 beats/min (W_{1-0}), as originally described by Sjostrand (14, 15) The correlation between W_{1-0} and other physiological characteristics, for example, maximal oxygen uptake, is very good, as has been reported (8)

A work test developed for military medical purposes is the maximal working capacity test, $PWC_{max}6'$ (17) The test takes on an average 6 minutes to perform and consists of the measurement of the longest time the individual can work on the bicycle ergometer at a standard load, usually 1400 kpm/min From this is calculated $PWC_{max}6'$, i e the heaviest load the individual could have worked at for a full 6 minutes period Anaerobic processes contribute to this value as well as aerobic processes. It has been shown that the correlation between W_{1-0} and $PWC_{max}6'$ is high (17)

The ability to perform brief work has been investigated to a much larger extent than prolonged work has. Therefore, it was felt of interest to investigate the relationship between the capacity for brief exercise and that for prolonged exercise

Material and Procedure

Up to the present time in Sweden a brief medical examination has been carried out on all young men at the registration for military service This is done at 18 years of age As part of a reorganization of the registration procedure, various medical tests, among others, have been carried out on some young men at the time of registration (12, 13) Thus, in the autumn of 1965 in one registration district, Halland, all conscripts for registration about 1500 men in all

underwent a 2 day examination (10) of those various factors which were thought to influence physical working capacity, including anthropometric, muscular and circulatory variables The first day's examination consisted of, e g, ECG and blood pressure registration, followed by a maximal work test (17) Thereafter, 63 consecutive volunteers were chosen from those who were able to complete 6 minutes of work at 1400 kpm/min Seven of these had a hemoglobin concentration of less than 13 g% and were not studied further On the following day the maximal working time for various submaximal loads was measured on the remaining 56 men Table I shows anthropometric and other data on this group For comparison, Table II shows the mean and one standard deviation (SD) of certain variables for all of the conscripts called up from that particular district From this it can be seen that anthropometric, muscular and circulatory variables average about the same values in the group of 56 investigated in detail and the total group consisting of all those called up at that time All of the 56 also had normal hemoglobin sedimentation rate and urine analysis

Methods

A *medical history* was taken of all *subjects* studied A *physical examination* was performed *Height* was measured to the nearest cm with the subject barefooted *Weight* was measured to the nearest kg with the subject wearing only shorts For evaluation of skeletal size, the *femoral condylar breadth* was determined with the "Taster circle", graded in 0 1 cm (5) *Vital capacity* was measured to the nearest 100 ml (ATPS) upon maximal expiration using a Godart Expirograph The largest of 3 attempts was the value recorded *Heart volume* was calculated from frontal sagittal plane and lateral (7×7 cm) chest films with the subject standing (11) *Isometric muscle strength* — hand grip, elbow flexion, and knee extension —was measured (17) *Pulse rate* at rest during, and at the end of exercise was taken from the ECG (Elema Mingograph, Type 42) The method for registering the *resting ECG* has been described previously (13) Pulse rate during exercise was registered from one or more of the CH leads (7) Before carrying out the *$PWC_{max}6'$ test* (17), *hemoglobin concentration sedimentation rate* and *urine analysis*, particularly for glucose and protein were carried out. From the history physical examination and Ecg was determined which of the subjects were to partipate in the $PWC_{max}6$ test which was then carried out between noon and 4 p m The subjects were instructed to continue cycling at the load of 1400 kpm/min until they were unable to continue themselves They were told that if they did not put out their best effort the test would be repeated The work tests were done on a bicycle ergometer (6)

On Day 2 the *maximal* duration of exercise at a *submaximal work load* was investigated on the 56 selected individuals who were again advised to work until they could continue no longer All submaximal work load testing was begun

From MMUC (Military Medical Examination Center) and the Department of Clinical Physiology Karolinska sjukhuset, Stockholm and the Department of Clinical Physiology, University of Goteborg Sweden

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Material and Procedure

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	PWC_{max}*		Submaximal test			
Pulse rate at rest (beats/min)	Performance time (seconds)	Final pulse rate (beats/min)	Load (kpm/min)	Pulse rate 10 (beats/min)	Final pulse rate (beats/min)	Duration (min)
77	360	207	1200	202	210	12
91	340	200	1200	202	202	11
68	308	183	1200	185	185	12
76	390	210	1200	200	205	16
61	360	190	1200	184	193	31
82	345	200	1200	195	195	11
73	352	200	1100	200	200	13
70	330	190	1100	174	186	30
76	390	185	1100	178	191	37
76	360	180	1100	168	180	36
67	360	200	1200	195	196	20
81	390	175	1100	173	173	12
78	360	200	1200	194	194	9
57	370	190	1200	192	192	11
53	370	180	1200	171	168	16
82	360	18[illegible]	1100	168	168	21
62	383	185	1000	158	161	25
86	360	200	1000	175	180	26
81	320	195	1000	178	186	30
99	330	189	1000	184	186	32
77	360	200	1000	190	198	30
84	360	196	1000	172	190	40
57	345	190	1000	187	185	20
54	390	185	1000	158	170	[illegible]0
85	360	200	1100	185	195	18
88	330	210	1000	195	202	30
73	360	187	1100	172	187	31
65	360	195	950	173	186	40
68	381	197	1100	185	190	20
78	339	195	950	164	190	60
67	340	184	950	176	183	60
68	360	180	1150	180	186	14
69	339	195	1150	185	192	20
85	360	200	1150	200	200	11
104	340	200	1150	197	197	10
91	320	190	950	168	177	40
99	360	186	950	168	183	39
76	360	200	1050	194	193	15
70	400	190	1050	176	185	18
78	320	190	950	185	197	50

Table I Anthropometric and other data in 56 subjects

Subject	Height (cm)	Weight (kg)	Right Femoral condylar breadth (cm)	Vital capacity (l)	Heart volume (ml)	Muscle strength, right: Hand grip (kp)	Elbow flexion (kp)	Knee extension (kp)
1	177	65	9.7	4.1	780	50	30	45
2	172	66	9.5	4.1	680	65	38	61
3	175	71	10.5	4.9	790	81	39	61
4	179	65	9.5	5.0	640	76	39	58
5	174	59	9.4	4.8	800	62	40	40
6	180	67	9.9	5.6	650	64	35	57
7	173	62	9.5	3.3	700	68	35	51
8	179	69	9.5	5.4	700	81	46	60
9	178	77	10.1	4.5	950	82	48	56
10	184	65	10.4	4.7	740	73	37	48
11	180	62	10.0	4.3	800	57	35	60
12	169	66	9.2	4.8	810	60	33	64
13	188	68	9.5	5.8	650	74	40	50
14	171	57	9.0	4.1	700	56	30	66
15	181	71	9.7	5.0	700	69	45	74
16	178	76	10.0	5.3	980	66	35	71
17	178	70	9.3	4.5	760	51	31	51
18	172	68	9.7	4.4	800	69	44	68
19	173	61	9.4	5.1	770	79	42	58
20	183	65	9.5	4.9	660	62	34	47
22	183	72	9.5	4.5	720	50	32	50
23	179	63	9.4	5.2	720	65	31	62
24	165	55	9.2	3.7	680	55	38	52
25	169	67	9.4	3.2	1000	64	46	54
26	182	60	8.9	3.8	740	52	28	45
27	175	59	9.1	5.0	840	54	30	48
28	176	59	9.7	4.3	810	55	36	44
29	175	63	9.5	4.8	840	58	33	42
30	173	62	8.7	4.2	830	66	39	74
31	175	65	9.4	4.8	840	64	44	65
32	179	63	9.8	6.4	800	62	40	63
33	172	57	9.5	3.9	650	57	31	46
37	178	79	9.7	4.4	900	84	42	65
38	172	61	9.4	4.9	790	57	35	46
39	185	76	9.8	5.8	900	82	45	50
40	170	64	9.5	4.6	730	65	43	62
41	173	62	9.1	4.2	770	58	42	57
43	165	58	8.9	3.9	820	51	41	54
44	170	61	9.3	4.6	840	59	39	50
45	181	67	9.7	5.0	830	80	44	66

Pulse rate at rest (beats/min)	PWC$_{max}$* Performance time (seconds)	PWC$_{max}$* Final pulse rate (beats/min)	Submaximal test Load (kpm/min)	Submaximal test Pulse rate 10 (beats/min)	Submaximal test Final pulse rate (beats/min)	Submaximal test Duration (min)
85	370	192	1050	182	185	30
88	390	186	950	156	160	32
74	360	185	950	147	160	32
83	390	174	1150	163	163	10
63	400	180	1050	160	168	14
68	380	195	950	160	175	60
76	356	180	1050	150	150	13
55	360	180	1150	180	180	10
76	395	195	1050	175	195	60
59	350	177	1050	166	167	14
59	360	185	1050	185	186	26
79	330	183	1050	175	166	60
66	370	180	1050	170	182	60
58	370	190	1150	178	188	22
59	390	180	1100	170	180	20
65	360	182	1000	166	168	30
74.0	359.9	190.3	1073	178.0	184.1	27.1
11.9	21.9	8.8	86	13.7	13.0	15.5
53—	308—	174—	950—	147—	150—	9—
104	400	210	1200	202	210	60

TABLE II Some anthropometric and other data in one registration district (Halland) 1965

	Height (cm)	Weight (cm)	Right Femoral condylar breadth (cm)	Vital capacity (l)	Heart volume (ml)	Muscle strength right: Hand grip (kp)	Muscle strength right: Elbow flexion (kp)	Muscle strength right: Knee extension (kp)	Pulse rate at rest (beats/min)	Final pulse rate at PWC (beats/min)	Performance time at PWC (seconds)
n	1313	1313	1313	1313	1313	1313	1313	1313	1264	1232	1232
x	175.1	66.1	9.67	4.59	768	72.8	36.0	52.1	78.3	190.3	388.9
SD	3.3	8.7	0.65	0.67	125	123	6.9	11.0	13.9	10.1	253.3

Subject	Height (cm)	Weight (kg)	Right Femoral condylar breadth (cm)	Vital capacity (l)	Heart volume (ml)	Muscle strength right: Hand grip (kp)	Elbow flexion (kp)	Knee extension (kp)
46	175	74	10 3	5 2	740	63	35	61
47	188	77	10 3	5 1	720	67	36	67
48	185	76	10 3	5 3	870	84	50	67
49	183	81	9 8	5 1	870	87	51	79
51	172	69	9 6	4 3	980	62	33	63
52	178	67	9 4	4 9	720	60	50	48
54	172	64	9 0	4 4	700	49	30	35
55	174	64	9 6	3 7	840	53	31	51
56	181	63	9 9	5 0	970	51	32	46
57	180	63	9 9	4 8	920	59	29	45
58	181	64	9 8	4 7	850	61	29	50
59	181	74	10 5	5 2	1050	77	43	61
60	188	69	9 5	4 8	860	68	31	50
61	170	51	9 2	4 9	640	57	28	53
62	177	65	9 6	5 2	850	80	39	64
63	182	76	10 2	4 3	900	70	34	50
$\bar{x}$	176 9	66 1	9 60	4 7	796	64 8	37 4	55 9
SD	5 5	6 4	0 41	0 6	100	10 4	6 2	9 5
range	165—188	51—81	8 7—10 5	3 2—6 4	640—1050	49—87	28—51	35—79

at about 8 a m The subjects were not fasting The work loads were varied irregularly from individual to individual For purposes of calculating the results, all 56 subjects have been considered to have a $PWC_{max}6'$ of approximately 1400 kpm/min For the submaximal tests, the exercise times have been rounded off to the nearest whole minute For lack of time all submaximal tests have been automatically stopped at 60 minutes For analysis of the results, conventional statistical methods have been used (16)

Results

In addition to the anthropometric and other data, Table I presents the duration of the exercise at maximal and sub maximal loads together with the pulse rate at the end of the exercise It also shows the work load and pulse rate after 10 minutes work[1] of the submaximal exercise period The average duration of

[1] For subject 13 who reached 9 minutes at the submaximal test the pulse rate at the end of the exercise period has also been used as the 10 minute value

31 3 (SD ± 8 4 20—50) and 45 9 (SD ± 11 8, 32—60)

With regard to differences in duration of exercise between adjoining groups, that found for the group working at 950 kpm/min 45 9 min, is significantly longer than that at 1000 kpm/min, 31 3 min (P < 0 01) The differences in times between the 1150 kpm/min group, 13 9 min and the 1100 kpm/min group, 23 8 min, is also significant (P < 0 02) Because of the lack of significant dif ference in the duration of exercise be tween the other adjoining groups and because of the small size of the groups the material was com bined into 3 groups as follows I (work load 1200—1150) II (work load 1100—1050) and III (work load 1000—950) There was no significant difference between these 3 groups with respect to height weight skeletal size various measurements of muscle strength vital capacity resting pulse rate or pulse rate during exercise With respect to heart volume there was a significant difference between group I 752 ml and group II 842 ml (P < 0 01)

Since 2 points were measured for each individual one being 1400 kpm/min and 6 minutes it was possible to calculate a regression coefficient or slope of the individual load — log work time re lationship in the used coordinate system As seen in Fig 1 there is a wide scatter of such slopes because of the differences in durations of exercise at the submaxi mal tests For the 3 groups the mean slopes, with a minus sign, were I 621 (280—1136), II 543 (350—1043) and III 541 (434—765)

The mean pulse rate at the end of the submaximal test, 184 1 beats/min, was lower than that at the end of the maximal test, 190 3 beats/min, (P < 0 001) The pulse rate after 10 minutes of submaximal exercise, caculated in per cent of the highest pulse rate during the maximal or the submaximal test, is inversely correlated to the duration of exercise of the submaximal test (r = — 0 59 P < 0 001)

To obtain a better and a poorer' grouping as to capacity for submaximal exercise the material has been divided in the following manner Into the bet ter group have been put all who cycled at least 15 min at a level of 1200 kpm/ min. For loads of 1150 1100, 1050, 1000, and 950 kpm/min the corresponding dividing line was set at 18 5 22 25 5, 35, and 45 min respectively In this way the better group included 21 persons, and the poorer group 35 The mean performance time at the maximal test for the 2 groups was 361 1 (SD ± 21 7) and 359 1 (SD ± 22 3) sec for the better and poorer group respectively Table III shows anthropometric and other data for this manner of grouping the subjects and indicates that for these variables the 2 groups were not signifi cantly different

If the work time really found at the $PWC_{max}6$ test is used for calculation instead of 6 min in those cases it was not exactly 360 seconds, the mean slope values for groups I II and III are 635 552 and 534 Between these mean slope values and the corresponding values mentioned above there are no significant differences (P > 0 2)

Discussion

Fifty six healthy young men all of whom were able to cycle approximately 6 min utes at a load of 1400 kpm/min, i e

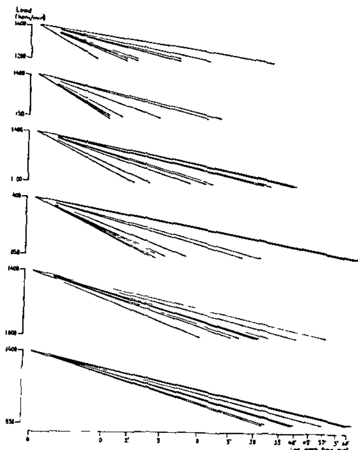


Fig 1
Work loads and work times at 2 tests in 56 subjects (6 groups) Each line represents one subject

exercise at 1400 kpm/min was 359 9 sec (SD ± 21 9, range 308—400), which corresponds to a $PWC_{max}6'$ value of 1399 9 kpm/min (SD ± 17 5, range 1350—1430)

The submaximal tests were carried out at one of 6 loads Ten subjects were tested each at loads of 1200, 1100, 1050 or 1000 kpm/min Seven subjects were tested at 1150 kpm/min and nine at 950 kpm min Figure 1 shows graphically the results of the two work tests carried out on each of the 56 subjects For practical reasons the first value for all has been set at 6 min for a work load of 1400 kpm/min The calculated mean $PWC_{max}6'$ values in the 6 groups with submaximal work loads from 1200 to 950 kpm/min were respectively 1397 (SD ± 19), 1400 (SD ± 14) 1406 (SD ± 15) 1408 (SD ± 17), 1395 (SD ± 18) and 1393 (SD ± 20) kpm/min On the ordinate are plotted the levels of the maximal and submaximal work loads, and on the abscissa the logarithm of the durations of exercise A logarithmic abscissa has been used, since with a semilogarithmic system, the individual relationship between work load and duration of exercise displays a characteristic form (1) The mean durations of exercise in minutes for the groups with loads from 1200 kpm/min to 950 kpm/min are 14 9 (SD ± 6 5, 9—31), 13 9 (SD ± 5 1, 10—22), 23 8 (SD ± 9 1 12—37), 31 0 (SD ± 20 7, 13—60)

31 3 (SD ± 8 4, 20—50) and 45 9 (SD ± 11 8 32—60)

With regard to differences in duration of exercise between adjoining groups, that found for the group working at 950 kpm/min 45 9 min, is significantly longer than that at 1000 kpm/min, 31 3 min ($P < 0 01$) The differences in times between the 1150 kpm/min group, 13 9 min, and the 1100 kpm/min group, 23 8 min, is also significant ($P < 0 02$) Because of the lack of significant difference in the duration of exercise between the other adjoining groups and because of the small size of the groups the material was combined into 3 groups, as follows I (work load 1200—1150) II (work load 1100—1050) and III (work load 1000—950) There was no significant difference between these 3 groups with respect to height weight skeletal size various measurements of muscle strength vital capacity resting pulse rate or pulse rate during exercise With respect to heart volume there was a significant difference between group I 752 ml and group II 842 ml ($P < 0 01$)

Since 2 points were measured for each individual one being 1400 kpm/min and 6 minutes it was possible to calculate a regression coefficient or slope of the individual load — log work time relationship in the used coordinate system As seen in Fig 1 there is a wide scatter of such slopes because of the differences in durations of exercise at the submaximal tests. For the 3 groups the mean slopes, with a minus sign were I 621 (280—1136), II 543 (350—1043) and III 541 (434—765)

The mean pulse rate at the end of the submaximal test, 184 1 beats/min, was lower than that at the end of the maximal test 190 3 beats/min, ($P < 0 001$) The pulse rate after 10 minutes of submaximal exercise, caculated in per cent of the highest pulse rate during the maximal or the submaximal test, is inversely correlated to the duration of exercise of the submaximal test ($r = -0 59$ $P < 0 001$)

To obtain a better and a poorer grouping as to capacity for submaximal exercise the material has been divided in the following manner Into the better group have been put all who cycled at least 15 min at a level of 1200 kpm/min For loads of 1150 1100 1050, 1000, and 950 kpm/min the corresponding dividing line was set at 18 5 22 25 5 35 and 45 min respectively In this way the better group included 21 persons, and the poorer group 35 The mean performance time at the maximal test for the 2 groups was 361 1 (SD ± 21 7) and 359 1 (SD ± 22 3) sec for the better and poorer group respectively Table III shows anthropometric and other data for this manner of grouping the subjects and indicates that for these variables the 2 groups were not significantly different

If the work time really found at the $PWC_{max}6$ test is used for calculation instead of 6 min in those cases it was not exactly 360 seconds, the mean slope values for groups I II and III are 635 552 and 534 Between these mean slope values and the corresponding values mentioned above there are no significant differences ($P > 0 2$)

Discussion

Fifty six healthy young men all of whom were able to cycle approximately 6 minutes at a load of 1400 kpm/min, re

TABLE III Some anthropometric and other data in 'better' (n=21) and 'poorer' (n=35) groups

Variable	'better' $\bar{x}$	group SD	'poorer' $\bar{x}$	group SD	Significance of differences (p) between groups
Height (cm)	178.4	4.3	176.1	6.0	>0.1
Weight (kg)	66.3	6.5	65.9	6.5	>0.2
Fem cond breadth (right) (cm)	9.73	0.36	9.53	0.42	>0.05
Vital capacity (l)	4.84	0.59	4.60	0.61	>0.1
Heart volume (ml)	812	114	787	91	>0.2
Muscle strength, right					
Handgrip (kp)	67.2	9.7	63.4	10.7	>0.1
Elbow flexion (kp)	39.1	5.8	36.4	6.5	>0.1
Knee extension (kp)	56.4	8.6	55.6	8.6	>0.2
Pulse rate at rest (beats/min)	70.1	9.3	76.3	12.8	>0.05
Final pulse rate at $PWC_{max}6'$ (beats/min)	189.9	7.4	190.6	9.6	>0.2
Final pulse rate at submax test (beats/min)	186.0	9.9	183.0	11.6	>0.2
Pulse rate 10 (beats/min)	176.5	10.4	178.9	15.3	>0.2
Pulse rate 10 in % highest pulse rate	92.6	4.5	93.6	5.5	>0.2

they had about the same $PWC_{max}6'$ value of 1400 kpm/min, have been studied with regard to the duration of exercise possible at a submaximal work load As shown in Table I and Fig 1 there is a clear scattering of individuals cycling at the same submaximal work load, despite approximately the same working capacity at 1400 kpm/min Our results show that there is a low correlation between the ability to perform maximal work of brief and long duration It does not seem to be a narrow direct relationship between these two abilities

With the type of investigation carried out here motivation plays a large role. The motivation of the subject is thus a prerequisite for obtaining optimal information That motivation was high in the present study, however, is indicated both by the high pulse rate at maximal and submaximal work and by the subjective impressions of the investigators In addition, the observations on economically well remunerated research subjects (2) speaks against lack of motivation as the single cause of low correlation between capacity for brief and prolonged exercise It is thus shown in that study (2) that capacity for prolonged work is correlated to body size to a lesser degree than capacity for brief work That capacity for brief exercise i e of some minutes duration is correlated to body size, among other things,

is well established (8) Division of the subjects in the present study into a 'better' and a 'poorer' group, based on duration of exercise at a submaximal load does not show any differences between the groups as to anthropometric or other variables Even though individual differences due to motivation cannot be completely ruled out, it should be noted that there was a great variation in working capacity at the submaximal load despite both the homogenity of the material and the uniformity of working capacity at the maximal test

That muscle glycogen even in humans is a necessary fuel for muscular work at various loads has been shown by a direct biopsy method (3 4) It has been shown that there is a great variation in muscle glycogen content in normals (9) The observed variations in working capacity at the submaximal load might therefore well be explained by differences in individual muscle glycogen content The duration of the maximal test is influenced by circulatory factors, in this respect the material is uniform The duration of the submaximal test is also to some extent dependent on circulatory factors but mostly upon the muscle glycogen content From this one might conclude that those subjects who were able to work for a longer time submaximally should have had a higher initial muscle glycogen content

Partly due to the experimental conditions the higher work loads at the submaximal test seem to give more information in relation to how long time they take as to capacity for prolonged submaximal work at the lower work loads there is a tendency for the best suited to prolonged work to be able to work a very long time Since it is of interest to predict both which are the best prolonged work subjects as well as how much work they can perform, it appears rational to use as short a prolonged capacity test as possible From the data of the present study, it seems that a work load difference even as little as 200 kpm/min below the maximal level could give information on which of those who have the same capacity for brief work have the best capabilities for prolonged work When these individuals' data are plotted in a diagram of the work load vs log of duration of work, it will be seen that they have numerically low regression coefficients or slopes in the regression equation as an expression of the individual relationship between work load — duration of work As previously reported (1) the practical value of this is that circulatory factors play a minor role the longer a prolonged work continues

Naturally the determination of the individual slope from only two points is affected with uncertainty Especially when the submaximal test was performed on high loads the scattering of slopes is large at lower loads the scattering is smaller However the mean slopes in groups I II and III are steeper than the corresponding value in a report where each subject had been tested more than twice and for longer work periods (1) That material (1) however, was older than the subjects in this study The 18 year old men also had lower mean values as to anthropometric and other comparable variables

As has been reported there is a good

TABLE III Some anthropometric and other data in ' better" (n=21) and ' poorer ' (n=35) groups

Variable	'better' $\bar{x}$	group SD	"poorer' $\bar{x}$	group SD	Significance of differences (p) between groups
Height (cm)	178 4	4 3	176 1	6 0	>0 1
Weight (kg)	66 3	6 5	65 9	6 5	>0 2
Fem cond breadth (right) (cm)	9 73	0 36	9 53	0 42	>0 05
Vital capacity (l)	4 84	0 59	4 60	0 61	>0 1
Heart volume (ml)	812	114	787	91	>0 2
Muscle strength right					
Handgrip (kp)	67 2	9 7	63 4	10 7	>0 1
Elbow flexion (kp)	39 1	5 8	36 4	6 5	>0 1
Knee extension (kp)	56 4	8 6	55 6	8 6	>0 2
Pulse rate at rest (beats/min)	70 1	9 3	76 3	12 8	>0 05
Final pulse rate at $PWC_{max}6'$ (beats/min)	189 9	7 4	190 6	9 6	>0 2
Final pulse rate at submax test (beats/min)	186 0	9 9	183 0	14 6	>0 2
Pulse rate 10 (beats/min)	176 5	10 4	178 9	15 3	>0 2
Pulse rate 10 in % highest pulse rate	92 6	4 5	93 6	5 5	>0 2

they had about the same $PWC_{max}6'$ value of 1400 kpm/min, have been studied with regard to the duration of exercise possible at a submaximal work load As shown in Table I and Fig 1 there is a clear scattering of individuals cycling at the same submaximal work load, despite approximately the same working capacity at 1400 kpm/min Our results show that there is a low correlation between the ability to perform maximal work of brief and long duration It does not seem to be a narrow direct relationship between these two abilities

With the type of investigation carried out here, motivation plays a large role The motivation of the subject is thus a prerequisite for obtaining optimal information That motivation was high in the present study, however, is indicated both by the high pulse rate at maximal and submaximal work and by the subjective impressions of the investigators In addition, the observations on economically well remunerated research subjects (2) speaks against lack of motivation as the single cause of low correlation between capacity for brief and prolonged exercise It is thus shown in that study (2) that capacity for prolonged work is correlated to body size to a lesser degree than capacity for brief work That capacity for brief exercise i e of some minutes duration, is correlated to body size, among other things,

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correlation between individual muscle glycogen content and capacity for prolonged exercise (3, 4) However, a biopsy technic seems unsuitable for prediction of an individual's capacity for prolonged exercise, for example, in investigations carried out at the time of military registration A practical method for prediction of working capacity of maximal work during long time is thought to be a testing procedure with 2 tests, one maximal, with performance time 2—12 minutes and one submaximal, which will take into account several determining factors With such a testing procedure one would appear to have a practical means of determining those in a group best capable of prolonged exercise

Summary

In connection with medical tests performed during 2 days at the time of registration for military service, an investigation was carried out on approximately 1500 18 year old men in one registration district Anthropometric, circulatory and muscular variables, were recorded After testing the maximal working capacity, $PWC_{max}6'$, 56 subjects were selected all of whom at a work load of 1400 kpm/min had a maximal duration of work of approximately 6 min It was shown that these 56 were an homogeneous group with respect to other variables too Pulse rate at the end of $PWC_{max}6'$ averaged about 190 beats/min On Day 2 the maximal duration of work was investigated at one of the following submaximal work loads 1200, 1150 1100, 1050 1000 and 950 kpm/min It was shown that there was a very wide scatter of work durations at these loads The observed results were discussed A testing procedure to establish the capacity of an individual to perform prolonged work is suggested

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ical working capacity may thus be defined as the work intensity that a subject can perform at a given pulse rate, for instance 130 or 170 beats/min or at an oxygen uptake of for instance 50 or 80 per cent of the maximal oxygen uptake, or at a given rating value of a rating scale of perceived exertion

For inter individual comparisons it is however important to know how age may influence such submaximal measures of physical working capacity that are based on pulse rate and rating of perceived exertion Several authors have found that the maximal pulse rate is on an average lower in a group of old subjects than in young ones (1, 9 12 14) It therefore seemed of interest to study the relationships between pulse rate, work intensity and the subjects perception of exertion in various age groups For this purpose we thought it particularly valuable to include in the study a group of subjects of various ages who performed a similar type of physical work in their daily activities (lumber workers)

Material

Only subjectively healthy individuals were included in the investigation ECG was recorded on all subjects at rest during and after exercise on a bicycle ergometer and blood pressure was measured at rest in all subjects and in most cases also during exercise Excluded were all subjects with high blood pressure at rest (systolic > 160, diastolic > 90 mm Hg) or during exercise (systolic > 260 mm Hg) and subjects with pathological ECG changes at rest or during and after exercise indicating coronary insufficiency Excluded were also subjects who complained of pain in the back or in the knee joint during exercise

Lumber workers This group consisted of 61 permanently employed male lumber workers (cutters) (Mo and Domsjo Ltd) They used motor saws for tree cutting and the rest of the work was manual Their work was physically heavy and the type of work had been the same for several years before the investigation started The workers volunteered for the examinations Salary and subsistance allowances were granted by the company and most subjects called upon took part in the examination The group is partially the same as that earlier described by Borg (3) The median age was 45 years and the range 27—63 years

The 61 lumber workers were obtained from a group of 81 lumber workers, by excluding 10 subjects who had arterial hypertension or signs of coronary insufficiency, and 10 subjects because of pains in the back or the legs during exercise The group of 81 lumber workers were representative for lumber workers of this area according to their type of work and income

Mixed material This was a professionally heterogenous group of male subjects The habits of daily life varied in contrast to the comparatively homogenous habits of the lumber workers The material was not sampled in order to make the various age groups random samples of the population However, the physical working capacity PWC_{170} of the various age groups (see results) agreed fairly well with that of other similarly sampled

From the Departments of Clinical physiology and Clinical psychology, University of Umeå, Sweden

Perceived Exertion and Pulse Rate during Graded Exercise in Various Age Groups

By

G. Borg and H. Linderholm

The absolute work intensity that a subject performs is usually estimated from the oxygen uptake, or measured directly as the external work performed on an ergometer. If work is done on a bicycle ergometer there is a close relationship between the rate of oxygen uptake and the external work load (work intensity) due to the quite constant mechanical efficiency in this type of work (13, 15 and others).

The relative work intensity of an individual, i.e. the work intensity he performs in relation to his maximal capacity can be estimated from the oxygen uptake in relation to his maximal oxygen uptake capacity. The pulse rate during exercise can also be used as a measure of the relative work intensity. This estimate is based on the fairly linear relationship between pulse rate and work load (5) and the rather uniform range of pulse rates between individuals, i.e. the range from the pulse rate at rest to the maximal pulse rate during exercise, at least in a homogenous age group (15).

In a similar way as pulse rate, the subjects perception of exertion during exercise may be used to indicate the relative work intensity. A relationship between pulse rate and the degree of perceived exertion seems to be generally accepted, and a work load that gives a pulse rate of 150—175 beats/min, i.e. fairly close to the maximal pulse rate has been regarded as very heavy work, while a work load giving a pulse rate of 75—100 beats/min has been looked upon as light exercise (6). This concept has recently been confirmed. In a homogenous group of young healthy men a correlation coefficient of 0.83 was found between the values obtained with a rating scale of subjective exertion and the pulse rate during exercise of various intensity (3).

When comparing the working capacity between individuals, various tests of maximal working capacity may be used. Often submaximal tests are preferred. Then, at a given level of relative work intensity the work load is compared between individuals. A submaximal phys

most subjects had a good motivation to do their best. The test leader was somewhat cautious in encouraging the oldest subjects to do their utmost

Blood pressure was measured according to Riva Rocci (2)

Evaluation of physical working capacity Submaximal measures of physical working capacity were determined from the pulse rate at known work intensities as follows The work load on the bicycle ergometer (kpm/min) corresponding to a pulse rate of 130 or 170 beats/min was estimated by linear inter or extrapolation in a pulse rate — work load diagram ' and was used as a measure of physical working capacity (PWC_{130} and PWC_{170}) of an individual (10, 11, 13)

In a similar way measures of physical working capacity were estimated from the rating of perceived exertion by linear interpolation (not extrapolation) in the rating value work load diagram making use of the fairly linear relationship

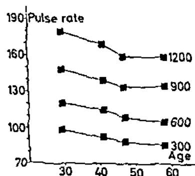


Figure 1 a Lumber workers (■)

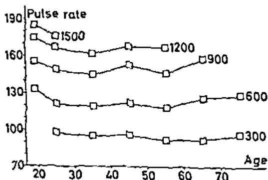


Figure 1 b Mixed material (□)

Figs 1 a and 1 b Mean pulse rate in beats per minute in relation to age at given work loads (300—1500 kpm/min)

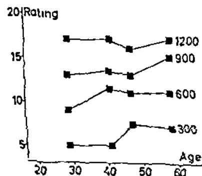


Figure 2 a Lumber workers

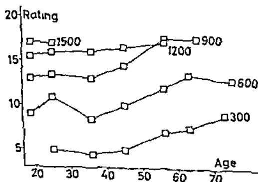


Figure 2 b Mixed material

Figs 2 a and 2 b Medians of rating values in relation to age at given work loads (300—1500 kpm/min)

groups (8, 12) The mixed material included the following age groups

a) *Group 18—20 years of age* This group consisted of conscripts, who were examined during the first week of their military service Due to the way in which they were selected by the military authorities they may be regarded as a comparatively representative group of healthy Swedish men, 18—20 years of age They were ordered to take part in the examination which was part of a regular check on their physical performance

b) *Group 20—29 years of age* This group consisted mainly of students who volonteered or were paid for taking part in the examination

c) *Groups 30—39 and 40—49 years of age* consisted of members of a club for gymnastics, representing a wide variety of occupations, some students and teachers, all of them volunteered

d) *Groups 50—59, 60—69, and 70—79 years of age* consisted of apparently healthy people of various occupations who were examined at the hospital for health control

Methods

A work test according to Sjostrand (10) and Wahlund (13) was used The subject worked on a bicycle ergometer (7) The first load was 300 kpm/min The load was increased stepwise by 300 kpm/min The work on each load lasted for 6 minutes and the subjects worked until unable to continue or until signs or symptoms appeared, such as ECG changes, which required interruption of the exercise

Pulse rate ECG was recorded after 10 minutes rest in the supine position in most cases, during each work load and after the work test Pulse rates used were obtained from the ECG tracing recorded at rest, at the 6th minute of each load, and at the end of the work test

Evaluation of subjectively perceived exertion The subject was asked about the perceived exertion according to a rating scale between the 5th and 6th minute of exercise at each work load and usually near the end of the work test The rating scale consisted of 21 grades where all the odd scale values from 3 to 19 were anchored with the aid of verbal expressions The latter were chosen so that the scale should receive a good interindividual validity, i e only well known terms with a comparatively constant meaning were chosen The scale was presented to the subjects in quarto format with equal distances between the figures and in the following terms 3 Extremely light, 5 Very light, 7 Light, 9 Rather light, 11 Neither light nor laborious, 13 Rather laborious, 15 Laborious, 17 Very laborious, 19 Extremely laborious (3)

Maximal pulse rate The highest pulse rate during the work test was recorded in order to compare it with the maximal pulse rate found in various age groups by other authors This was done with the intention of getting some indication that the subjects worked close to their maximal working capacity, when the work is generally perceived as extremely laborious The blood lactate concentration as a check of maximal exertion was not determined during or after the work According to the test leader there are reasons to believe, however, that

most subjects had a good motivation to do their best The test leader was somewhat cautious in encouraging the oldest subjects to do their utmost.

Blood pressure was measured according to Riva Rocci (2)

Evaluation of physical working capacity Submaximal measures of physical working capacity were determined from the pulse rate at known work intensities as follows The work load on the bicycle ergometer (kpm/min) corresponding to a pulse rate of 130 or 170 beats/min was estimated by linear inter or extrapolation in a pulse rate — work load diagram and was used as a measure of physical working capacity (PWC_{130} and PWC_{170}) of an individual (10 11, 13)

In a similar way measures of physical working capacity were estimated from the rating of perceived exertion by linear interpolation (not extrapolation) in the 'rating value work load diagram making use of the fairly linear relationship

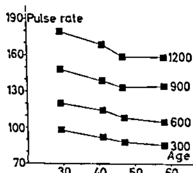


Figure 1 a Lumber workers (■)

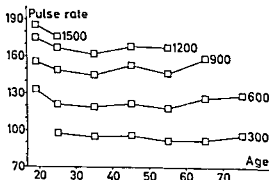


Figure 1 b Mixed material (□)

Figs 1 a and 1 b Mean pulse rate in beats per minute in relation to age at given work loads (300—1500 kpm/min)

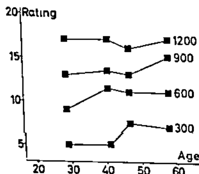


Figure 2 a Lumber workers

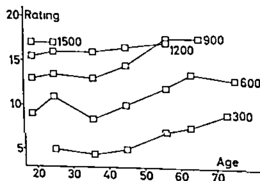


Figure 2 b Mixed material

Figs 2 a and 2 b Medians of rating values in relation to age at given work loads (300—1500 kpm/min)

TABLES I a and I b The tables give the mean pulse rate in beats per minute (M) with the standard ... 600 etc) in different age groups n = number of subjects examined

a Lumber workers

	Age 27—34				Age 35—44			
		pulse rate		R		pulse rate		R
Work load	n	M	±S D	Md	n	M	±S D	Md
0	8	71	±11 2		17	66	±10 4	
300	8	98	±16 5	5 0	17	92	± 6 7	5 0
600	8	120	±18 3	9 0	17	114	±14 4	11 5
900	8	148	±24 4	13 0	17	139	±14 5	13 5
1200	8	179	± 8 5	17 0	17	168	±14 1	17 0

b Mixed material

	Age 18—20				Age 20—29			
		pulse rate		R		pulse rate		R
Work load	n	M	±S D	Md	n	M	±S D	Md
0					58	69	±13 0	
300	61	133	±16 7	9	65	98	±13 3	5
600	73	156	±16 8	13	70	121	±14 9	11
900	69	175	±13 5	15 5	73	149	±18 9	13 5
1200	41	185	± 9 6	17	59	167	±13 5	16
1500					13	180	±11 9	17

Work load	Age 50—59				Age 60—69			
		pulse rate		R		pulse rate		R
	n	M	±S D	Md	n	M	±S D	Md
0	13	69	± 8 5		14	73	±11 5	
300	15	92	±13 6	7	14	92	±11 0	7 5
600	15	118	±13 5	12	14	126	±13 3	13 5
900	15	145	±18 4	17 5	4	158	± 5 3	
1200	6	167	±13 2	18				

between rating value and work load (between R_9—R_{17}) The rating values R_{13} and R_{17} were used as reference levels giving the measures of physical working capacity PWC_{R13} and PWC_{R17}

Results

The pulse rate and the rating of per

deviation (S D) and the median rating value R (Md) for different work loads (300

Age 45—54

	pulse rate		R
n	M	±S D	Md
27	66	±14 3	
27	88	±13 4	7 5
27	108	±15 1	11 0
27	133	±18 1	13 0
27	158	±14 1	16 0

Age 55—63

	pulse rate		R
n	M	±S D	Md
9	61	± 8 7	
9	85	± 8 2	7 0
9	105	± 7 9	11 0
9	134	± 9 5	15 0
9	158	±13 6	17 0

Age 30—39

	pulse rate		R
n	M	±S D	Md
24	70	± 9 7	
26	95	±13 2	4 5
26	119	±13 2	8 5
26	*145*	±17 1	13
18	162	±12 1	16

Age 40—49

	pulse rate		R
n	M	±S D	Md
7	72	±14 5	
9	96	±11 6	5
9	122	±13 8	10
9	153	±17 5	14 5
6	168	±10 7	16.5

Age 70—79

	pulse rate		R
n	M	±S D	Md
5	62	± 9 6	
6	96	±27 8	9
6	128	±33 1	12 5

ceived exertion (R) increased rather linearly with increasing work load in all the examined groups (Table I) When only a few subjects of a group managed to perform work on the highest work loads these results were not included in the table

In the group of lumber workers the

Table II The correlation coefficients (r) with significance levels (P) for the relationship between pulse rate at different given work loads and age in the groups of lumber workers The age in this group was rather normally distributed

Work load	r	P
300	—0 35	<0 01
600	—0 33	<0 01
900	—0 26	<0 05
1200	—0 41	<0 01

Table III Test of the statistical significance of the difference in rating at 600 kpm/min with age in the mixed material The material is divided above and below the total median in rating and above and below 50 years of age n = 139

	Rating		
Age	Below Md	Above Md	Total
Below 50 years	62	43	105
Above 50 years	9	25	34
Total	71	68	139

Chi square = 10, 1 P < 0 01

pulse rate at a given work load decreased with age (Table I, II and Fig 1a) In the 'mixed material the pulse rate at a given work load was quite constant (Fig 1b)

The rating of perceived exertion at a given work load is shown by Table I and Fig 2a and b In the group of lumber workers the rating at a given work load

Tables IV a and IV b The tables give the mean pulse rate in beats per minute (M) with the standard deviation (S D) for different ratings

a Lumber workers

	Age 27—34		Age 35—44	
Rating	n	M±S D	n	M±S D
9	8	119±20 6	15	116±15 6
11	8	135±25 2	17	126±22 6
13	8	151±23 0	17	141±22 9
15	8	167±17 4	16	158±22 7
17	7	179±12 7	14	169±20 7

	Age 45—54		Age 55—63	
Rating	n	M±S D	n	M±S D
9	23	102±13 9	7	97±12 0
11	27	115±18 8	9	106±16 8
13	26	131±20 3	9	124±20 1
15	23	147±22 0	7	134±13 3
17	17	158±21 6	4	150±17 5

was almost unchanged with increasing age while there was a significant increase in the mixed material (Table III)

At a given rating of perceived exertion the pulse rate decreased with increasing age in the group of lumber workers and in the mixed material (Fig 3a and b and Table IVa and b) Table V shows that there was a statistically significant negative correlation between pulse rate at a given rating value and age, particularly for low rating values in the group of lumber workers

In the mixed material the age group 20—39 years was compared with the age groups 50—79 years At given ratings of perceived exertion, R_9, R_{13} and R_{17} the pulse rate was statistically signif

b Mixed material

Rating	Age 18—20 n	Age 18—20 M±S D	Age 20—29 n	Age 20—29 M±S D	Age 30—39 n	Age 30—39 M±S D	Age 40—49 n	Age 40—49 M±S D
9	47	140±18 6	65	166±15 0	26	118±17 2	9	117±14 9
11	67	148±20 2	72	127±16 2	25	128±14 1	9	129±16 5
13	72	159±18 9	72	143±18 8	25	144±16 8	9	144±15 8
15	72	170±15 0	67	158±16 8	20	155±16 5	8	159±14 8
17	69	181±14 1	44	170±13 4	8	158±11 8	4	162±15 6

Rating	Age 50—59 n	Age 50—59 M±S D	Age 60—69 n	Age 60—69 M±S D	Age 70—79 n	Age 70—79 M±S D
9	7	99±17 1	9	105±18 3	4	104±29 8
11	15	113±16 1	14	113±17 5	5	117±24 2
13	14	130±18 1	14	126±18 8	4	122±17 8
15	13	142±17 4	11	139±16 9	4	136±16 3
17	8	146±13 3	7	152±11 1	2	143± 6 4

TABLE V Correlation coefficient (r) with levels of significance (P) for the relationship between age and pulse rate for *different rating values (R)* in the group of lumbar workers

R	r	P
9	−0 50	<0 01
11	−0 43	<0 01
13	−0 29	<0 05
15	−0 26	<0 05
17	−0 21	>0 05

icantly higher in the young age group ($p < 0.01$) The change with age in the relationship between pulse rate and rating of perceived exertion is also shown by *Fig 4a and b*

Mean values for measures of physical working capacity are given in Table VIa and b $PWC_{1,0}$ and $PWC_{1,6}$ increased slightly with increasing age in the group of lumber workers but was rather constant with increasing age in

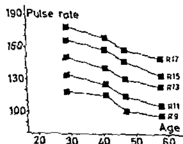


Figure 3 a Lumber workers

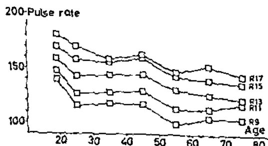


Figure 3 b Mixed material

Figs. 3 a and 3 b Mean pulse rate in relation to age at given rating values (R 9—R 17)

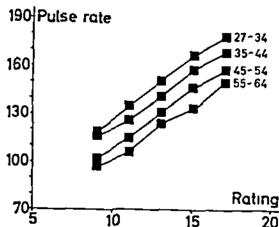


Figure 4 a Lumber workers

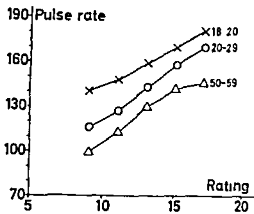


Figure 4 b Mixed material

Figs 4 a and 4 b Mean pulse rate in relation to ratings of perceived exertion

the mixed material except in the oldest age groups, 60—79 years, in which it decreased The physical working capacity estimated from the rating of perceived exertion PWC_{R13} and PWC_{R17} was rather independent of age in the group of lumber workers but decreased markedly in the mixed material with increasing age

The highest observed pulse rate in the different age groups was compared with the maximal pulse rates of com

TABLE VI a Mean (M) values and S D of maximal pulse rate and different measures of physical working capacity (PWC) in lumbar workers For definition of PWC_{130}, $PWC_{1\,0}$, PWC_{R13} and PWC_{R17} see under methods PWC_P is defined in the discussion, p 11 see also Table VII

Age groups		Max pulse rate	PWC_{130}	$PWC_{1\,0}$	PWC_{R13}	PWC_{R17}	PWC_P
27—34							
years	M	186	704	1085	922	1234	1025
n=8	S D	*7 0*	*195*	*149*	*83*	*91*	158
35—44							
years	M	178	786	1219	918	1279	1082
n=17	S D	10 2	152	172	222	225	167
45—54							
years	M	164	869	1271	897	1254	1088
n=27	S D	15 3	196	223	195	262	215
55—63							
years	M	150	868	1279	871	1122	1010
n=9	S D	15 2	99	104	208	189	102
Total	M	168	824	1233	893	1239	1066
n=61	S D	17 3	183	197	200	230	188

TABLE VI b Mean values of corresponding measures as in Table VI a for different age groups of the mixed material

Age in years	n	Max pulse rate	PWC$_{130}$	PWC$_{170}$	PWC$_{R13}$	PWC$_{R1}$	PWC$_{P}$
18—20	73	189	560	1120	960	1270	1130
20—29	73	179	720	1250	890	1250	1200
30—39	26	174	745	1250	920	1100	1130
40—49	9	172	636	1140	792	1193	950
50—59	15	166	735	1220	740	920	930
60—69	14	150	635	990	600	830	720
70—79	6	150	583	820	588	780	600

parable age groups reported by other authors (Fig 5) The decrease in the maximal pulse rate with increasing age is approximately equal in all the examined materials

Discussion

The results show that exercise at a given pulse rate is perceived to be heavier by old subjects than by young ones The relationship beween the physical working capacity estimated from the pulse rate and that estimated from the subjective rating of exertion was consequently found to change with age

The 61 lumber workers of various ages constituted a group of particular interest The daily physical activities of these workers in their heavy daily work were probably quite similar independent of age and most likely the workers were well adapted to this degree of physical activity In this group the physical working capacity estimated from rating of exertion, PWC$_{i\,13}$ and PWC$_{R17}$ was rather constant while the physical working capacity estimated from pulse rate,

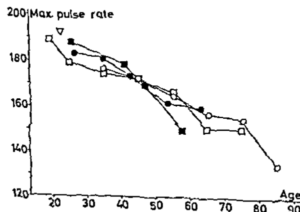


Fig 5 Maximal pulse rate in beats per minute *in relation to* age in different male groups Lumber workers, (■) mixed material, (□) material from Åstrand (14) (●) Åstrand (15) (▽) and Strandell (12) (○)

PWC_{130} and PWC_{170}, increased with age

In the mixed material PWC_{130} and PWC_{170} were rather constant while PWC_{P13} and PWC_{R17} decreased with increasing age The rather constant PWC_{130} and PWC_{170} in this group is in agreement with results obtained in rather similar groups of healthy subjects of various ages (8, 12) It is likely that the level of physical activity to which the old subjects of these groups were adapted was lower than that which the young subjects were used to The old subjects therefore are likely to be less physically fit than the young ones

It is known that the maximal pulse rate decreases with increasing age while the pulse rate at rest is rather constant, Table I and ref (9, 12, 14) For old individuals PWC_{170} can usually be obtained only by extrapolation as the recorded maximal pulse rate of such individuals during exhaustive exercise is often below 170 beats/min The results indicate that the physical working capacity estimated from the rating of exertion, for instance PWC_{R17}, measures a more constant fraction of the maximal working capacity in individuals of different age than PWC_{130} or PWC_{170}

If the pulse rate at the rating value R_{17} is plotted against age, Fig 3, the decrease in this pulse rate with increasing age compares fairly well with the decrease in maximal pulse rate with increasing age (Fig 5) The maximal pulse rate is, however about 10 beats/min higher than the pulse rate at R_{17}

In order to determine from pulse rate a submaximal work load that has the same meaning (the same relative work

TABLE VII Suggested reference levels in pulse rate for the calculation of physical working capacity (PWC_I) at different age PWC_I = the rate of work (work load) performed at the suggested pulse rate level

Age		Pulse rate reference level
Center	Interval	
20	19—23	*170*
	24—31	165
35	32—39	*160*
	40—46	155
50	47—53	*150*
	54—61	145
65	62—69	*140*
	70—76	135
80	77—83	*130*

load) independent of age it seems necessary to change the reference level in pulse rate for the age of the subject Such a reference level can be obtained if a constant fraction of the difference between a basal pulse rate at rest and the maximal pulse rate is added to the basal pulse rate If the constant fraction is 0 8 and the pulse rate at rest is 70 beats/min, then the reference level at the age 20 is 170, which is that commonly applied (10, 11, 13) Pulse reference levels for older age are proposed in Table VII They correspond to similar corrections that have been proposed earlier (1 14) Using such a correction to obtain a measure of the physical working capacity at a pulse rate that at various ages, respresents on an average the same relative degree of physical exertion, PWC_I, leads to quite a good

agreement between this measure and that estimated from a rating scale PWC_R (see Table VI a and b)

One for the present, commonly employed measure of submaximal working capacity, PWC_{170} is independent of the attitude and motivation of the tested subject However, the relationship between PWC_{170} and maximal performance varies with age PWC_{170} on the other hand is a measure of physical working capacity that seems to be almost independent of age in healthy subjects as suggested by our observations and the results of other authors (8 12, 14) It has also been used extensively in medical laboratory practise as a test to detect deviations from normal conditions in disease (10 11 13) For this purpose, PWC_{170} being a measure of the oxygen pulse and a relative measure of heart stroke volume has been found to be useful for comparison with measures of hemodynamic and body size characteristics (11) The PWC_P as suggested here as a measure of physical performance may become a useful complement however especially when making predictions of physical working capacity in practical situations

PWC_R seems to be well related to real physical performance (maximal working capacity) independent of age in healthy subjects However it is dependent on the attitude and motivation of the subjects and in psychological situations e g if special interests are involved the rating of an individual may become unreliable or an indicator of something else than physical exertion (4)

Summary

Pulse rate, rating of perceived exertion, and the highest pulse rate during a work test with stepwise increasing load on a bicycle ergometer until the subjects were exhausted were recorded in two groups of healthy adult male subjects covering a wide age range One group consisted of lumber workers This group was quite homogenous with regard to physical activity during daily work The other group was a professionally heterogenous mixed group in which the level of daily physical activity varied

In the group of lumber workers the pulse rate at the same work intensity decreased with increasing age while the rating value was approximately constant In the mixed group the pulse rate at the same work intensity was quite constant while the rating value increased with increasing age

In the two groups the pulse rate at equal rating decreased with increasing age There was an approximately linear relationship between pulse rate and rating value in the middle range of the rating scale The relationships between pulse rate and rating value were different in the various age groups In general with increasing age the work resulting in a given pulse frequency was rated to be more laborious

The highest observed pulse rate during exercise decreased with age in the two examined groups in a similar way as in groups observed by other authors It was found that the pulse rate at a given rating R_{17} = very laborious decreased in a similar way with age only that the pulse rate was about 10 beats/min lower than the highest pulse rates

PWC$_{130}$ and PWC$_{170}$, increased with age

In the mixed material PWC$_{130}$ and PWC$_{170}$ were rather constant while PWC$_{R13}$ and PWC$_{R17}$ decreased with increasing age. The rather constant PWC$_{130}$ and PWC$_{170}$ in this group is in agreement with results obtained in rather similar groups of healthy subjects of various ages (8, 12). It is likely that the level of physical activity to which the old subjects of these groups were adapted was lower than that which the young subjects were used to. The old subjects therefore are likely to be less physically fit than the young ones.

It is known that the maximal pulse rate decreases with increasing age while the pulse rate at rest is rather constant, Table I and ref. (9, 12, 14). For old individuals PWC$_{170}$ can usually be obtained only by extrapolation as the recorded maximal pulse rate of such individuals during exhaustive exercise is often below 170 beats/min. The results indicate that the physical working capacity estimated from the rating of exertion, for instance PWC$_{R17}$, measures a more constant fraction of the maximal working capacity in individuals of different age than PWC$_{130}$ or PWC$_{170}$.

If the pulse rate at the rating value R_{17} is plotted against age, Fig. 3, the decrease in this pulse rate with increasing age compares fairly well with the decrease in maximal pulse rate with increasing age (Fig. 5). The maximal pulse rate is, however, about 10 beats/min higher than the pulse rate at R_{17}.

In order to determine from pulse rate a submaximal work load that has the same meaning (the same relative work load) independent of age it seems necessary to change the reference level in pulse rate for the age of the subject. Such a reference level can be obtained if a constant fraction of the difference between a basal pulse rate at rest and the maximal pulse rate is added to the basal pulse rate. If the constant fraction is 0.8 and the pulse rate at rest is 70 beats/min, then the reference level at the age 20 is 170, which is that commonly applied (10, 11, 13). Pulse reference levels for older age are proposed in Table VII. They correspond to similar corrections that have been proposed earlier (1, 14). Using such a correction to obtain a measure of the physical working capacity at a pulse rate that at various ages, respresents on an average the same relative degree of physical exertion, PWC$_{P}$, leads to quite a good

TABLE VII. Suggested reference levels in pulse rate for the calculation of physical working capacity (PWC$_{P}$) at different age. PWC$_{P}$ = the rate of work (work load) performed at the suggested pulse rate level.

Age: Center	Age: Interval	Pulse rate reference level
20	19—23	*170*
	24—31	165
35	32—39	*160*
	40—46	155
50	47—53	*150*
	54—61	145
65	62—69	*140*
	70—76	135
80	77—83	*130*

agreement between this measure and that estimated from a rating scale PWC_R (see Table VI a and b)

One, for the present, commonly employed measure of submaximal working capacity PWC_{170} is independent of the attitude and motivation of the tested subject However, the relationship between PWC_{170} and maximal performance varies with age PWC_{170} on the other hand is a measure of physical working capacity that seems to be almost independent of age in healthy subjects as suggested by our observations and the results of other authors (8 12 14) It has also been used extensively in medical laboratory practise as a test to detect deviations from normal conditions in disease (10 11 13) For this purpose PWC_{170} being a measure of the oxygen pulse and a relative measure of heart stroke volume, has been found to be useful for comparison with measures of hemodynamic and body size characteristics (11) The PWC_P as suggested here as a measure of physical performance may become a useful complement however especially when making predictions of physical working capacity in practical situations

PWC_R seems to be well related to real physical performance (maximal working capacity) independent of age in healthy subjects However it is dependent on the attitude and motivation of the subjects and in psychological situations e g if special interests are involved the rating of an individual may become unreliable or an indicator of something else than physical exertion (4)

Summary

Pulse rate, rating of perceived exertion, and the highest pulse rate during a work test with stepwise increasing load on a bicycle ergometer until the subjects were exhausted were recorded in two groups of healthy adult male subjects covering a wide age range One group consisted of lumber workers This group was quite homogenous with regard to physical activity during daily work The other group was a professionally heterogenous mixed group in which the level of daily physical activity varied

In the group of lumber workers the pulse rate at the same work intensity decreased with increasing age while the rating value was approximately constant In the mixed group the pulse rate at the same work intensity was quite constant while the rating value increased with increasing age

In the two groups the pulse rate at equal rating decreased with increasing age There was an approximately linear relationship between pulse rate and rating value in the middle range of the rating scale The relationships between pulse rate and rating value were different in the various age groups In general with increasing age the work resulting in a given pulse frequency was rated to be more laborious

The highest observed pulse rate during exercise decreased with age in the two examined groups in a similar way as in groups observed by other authors It was found that the pulse rate at a given rating R_1 = very laborious , decreased in a similar way with age only that the pulse rate was about 10 beats/min lower than the highest pulse rates

Suitable measures of submaximal working capacity are discussed One such measure may be based on the rating of the perceived exertion When estimating a submaximal work capacity from pulse rate there are some advantages in using a fixed pulse rate, e g 170 as reference level For other purposes a pulse reference level that varies with age has advantages By choosing this level to correspond to the resting pulse rate with the addition of a fraction, e g 0 8, of the range between the pulse rate at rest and the maximal pulse rate, a measure of physical working capacity, PWC_P, may be obtained that coincides well with that based on rating of perceived exertion PWC_P may be assumed to be an approximately constant fraction of maximal working capacity independent of age

Acknowledgement

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From the Military Medical Examination Center (Swedish Defence Medical Board) and the Department of Clinical Physiology Karolinska Sjukhuset Stockholm

Physical working capacity, training and climate

By

Richard Hellstrom and Klas Linroth

A number of authors have previously studied in different contests, how different forms of physical training which engages important groups of muscles influence physical working capacity (9 13 14, 24) The effects on working capacity of the air temperature and humidity have also been studied (1 3 4 5 8 12, 25 26, 33 34) No studies however seem to have been published on changes in the working capacity of individuals who have lived for several months in a closed environment in which the essential variations have been in respect of training and climate The present paper reports such a study made during HMS *Alvsnabben* s 5 month (November—March) winter cruise from Sweden round South America and back 1957—58

Material

The study was conducted on 60 subjects 20 years of age serving on board HMS *Alvsnabben* Twenty were members of the regular ship s complement while forty were naval cadets The two groups were selected at random from among those assigned to this cruise. The cadets were further divided at random into two groups with twenty men in each This made it possible to study 3 groups which were trained in different ways One cadet group received special physical training, and this group was compared with the conscript crew members and with the other cadet group which received no physical training other than that included in the cadets regular programme One man in each of the groups fell out during the course of the investigation so that when the results were finally processed the groups contained 19 subjects each

Methods

Physical working capacity was investigated with a graded work test on a bicycle ergometer as originally described by Sjostrand (27) Working capacity is defined in this test as the intensity of work at a pulse rate of 170/min in steady state The tests were performed on an electric bicycle ergometer (10) which was carried on board throughout the cruise A control calibration when

the ship returned to Sweden showed no change compared with the initial readings

Before departure from Sweden, determinations were made of total heart volume in the recumbent position (17) and total Hb according to the CO-Hb method (28, 29)

Blood pressure was recorded with an ordinary blood pressure cuff, applied in all cases on the upper left arm with the subject sitting Owing to lack of time, the subjects did not rest prior to the examination Diastolic blood pressure has been taken when the sound began to fade

All determinations on board of body weight and height were made before lunch, at about 11 a m

Hb concentration was determined by the Sicca method

Muscular strength and breadth of the femoral condyle were measured in accordance with the methods reported by Lindegard (20, 21) The strength of the hands was thus determined three times in succession with a calibrated mechanical dynamometer, the highest reading being used

Procedure Having undergone relatively hard physical training at the Royal Swedish Naval College during the summer, the cadets were examined at Karolinska Hospital for the first time in August 1957 During September and October, prior to departure, they were engaged in demanding academic studies and examinations, and received little physical training

The crew members were first examined early in November, about 10 days before the ship left Sweden They had not undergone any very hard physical training

On board the *Älvsnabben*, the two cadet groups had the same instruction and duties The crew had less advanced instruction and otherwise took part in the general routine of the ship The three groups received different degrees of physical training throughout the cruise Group I (20 cadets) received extra physical training in addition to the regular programme, with its compulsory physical training The extra physical training consisted of gymnastics and cycle training The other 20 cadets (Group II) received no extra training but took part in the usual programme and received the ordinary physical training i e a light gymnastical practice The crew members (Group III) likewise received no extra physical training

The extra physical training given to Group I consisted until 15 January 1958 of 15—20 minutes of intensive morning gymnastics, on the average once or twice a week After 15 January, cycle exercises were also included on the average once a week for 15—20 minutes, with a check to ensure that the pulse rate reached at least 170 per minute

The three groups were examined once a month throughout the cruise usually over a period of 2—3 weeks The first batch of examinations was made in November 1957 and the last in March 1958, giving a total of 5 examinations for each group

Climate The ship encountered remarkably good weather throughout the cruise except for 2—3 days in the Caribbean Sea The study was thus not affected by sea conditions.

The temperature and relative humidity of the air varied within very wide limits but the transitions were gradual This means that each monthly examination was made and largely the same climatological conditions for the different groups When the ship left Sweden in November, the temperature was about + 6° centigrade and the relative humidity about 50 % In Buenos Aires during the first half of January it was + 38° in the shade with 98 % relative humidity Just over two weeks later the temperature in Punta Arenas was + 10° and the humidity 30—40 % The temperature in the ship and in the testroom varied between 18°—24°

Results

Some results from the examinations before and during the cruise are given in Tables I and II and Fig 1

The cadets had a relatively high physical working capacity (about 1200 kpm/min) in August after their summer training, but there was a considerable discrepancy between the two groups, in spite of the random division Those comprising Group I thus had about 100 kpm min. higher working capacity than those in Group II They were also found to be somewhat taller and heavier, and they had a higher value for total Hb and heart volume, although the difference was not statistically significant They also had a somewhat lower pulse rate at rest Thus the two cadet groups were not entirely comparable in respect of physical factors

The capacity of the cadets fell off statistically significantly between the examination in August and the first examination on board The decline was equally great in both groups and coincided with an intensive period of academic instruction

TABLE I Results obtained before the cruise

	Group I Aug		Group II Aug		Group III Nov	
	n	Mean	n	Mean	n	Mean
Age (years)	19	19.8	19	19.7	19	20.0
Height (cm)	19	178.8	19	178.4	19	175.6
Weight (kg)	19	70.3	19	66.6	19	67.3
Total Hb (g)	19	714	19	689	19	742
Hb conc ( )	19	97.9	19	93.4	19	96.9
Heart volume (ml)	19	891	19	810	19	791
Working capacity (kpm/min)	19	1268	19	1168	19	968
Pulse at rest (freq/min)	19	64.7	19	66.3	19	65.3
Blood pressure syst (mm Hg)	19	123.4	19	120.0	19	121.8
Blood pressure diast. (mm Hg)	19	86.1	19	83.4	19	80.3
Breadth of (right) femoral condyle[1] (cm)	16	9.5	17	9.4	16	9.4

[1] Determined immediately after the cruise

14 -4635 2 5 ppl 472

TABLE II Some data obtained during the cruise

	Nov		Dec		Jan		Feb		March	
	n	Mean	n	Mean	n	Mean	n	Mean	n	Mean
Group I										
Weight (kg)	19	71 5	19	70 5	19	70 3	19	70 3	19	70 7
Working capacity (kpm/min)	19	1016	19	1047	19	1047	19	1958	19	1116
Hand grip rt (kg)	19	53 6	19	57 1	19	57 4	19	57 6	19	56 8
Group II										
Weight (kg)	19	66 7	19	65 2	19	66 1	19	66 3	19	66 3
Working capacity (kpm/min)	19	953	17	888	19	974	19	926	19	989
Hand grip rt (kg)	19	51 4	18	53 2	19	55 2	19	54 9	19	54 7
Group III										
Weight (kg)	19	67 9	19	67 6	19	67 9	19	67 3	18	67 4
Working capacity (kpm/min)	19	1011	19	968	19	995	19	958	18	983
Hand grip rt (kg))	19	53 7	19	56 1	19	56 9	19	54 8	18	55 7

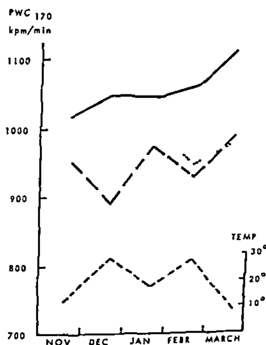


Fig 1 Physical working capacity and air temperature during HMS Alvsnabben s winter cruise in 1957—58 The figure shows the changes occurring during the voyage in the different groups physical working capacity in relation to the mean temperature of the air during the calendar month in question

——— Group I with extra physical training
— — — Group II with ordinary physical training
Group III with ordinary physical training
-- Air temperature (Monthly mean degrees in centigrades)

At the examination in November, 10 days before departure, the conscript members of the crew showed a relatively low physical working capacity (hardly 1000 kpm/min), considerably lower than that of the cadets in August, when the latter were well trained Heart volume, total Hb, weight and pulse at rest were of the same magnitude as for the two groups of cadets The conscripts however, were on average 3 cm shorter (Table 1)

At the first examination on board, in November, Groups I and III had the same mean physical working capacity, while that of Group II was somewhat lower

On board the *Älvsnabben*, working capacity developed differently among the two groups of cadets Group I that received extra physical training showed no decline in capacity in conjunction with variations in the air temperature Instead, their working capacity increased steadily and statistically significantly between the first and last examination on board Group II showed no improvement in working capacity the changes that did occur during the voyage resembled those recorded for the conscripts

The conscripts (Group III) showed a relatively low working capacity throughout the voyage with a number of minor variations Their capacity was somewhat greater when the air temperature was low and vice versa However none of the changes were significant

The muscular strength of the hand increased statistically significantly during the cruise in all three groups but most for the conscripts Body weight declined for all three groups, but significantly only for Group I (the cadets undergoing extra physical training)

The working capacity of the cadets was highest in August, when they were first examined Their capacity at that time was high in relation to total Hb In March, towards the end of the cruise, their working capacity was lower, although those undergoing extra physical training (Group I) had increased their capacity during the cruise The working capacity of the conscripts (Group III) was somewhat lower at the end of the cruise than in November of the previous year The difference, however, was not statistically significant

No statistically significant differences were recorded for blood pressure, Hb concentration or pulse at rest within or between the groups The small differences for these variables recorded at the five examinations did not show any trend to vary with temperature

Discussion

The physical working capacity of Groups I and II was high in relation to total Hb in August Such a relation between the physical working capacity and total Hb has been found to be common in individuals with a high physical working capacity (9) Group III, with a low working capacity in November, before the cruise showed a fairly ordinary relation between capacity and total Hb

The working capacity of the cadets (Groups I and II) declined significantly between the first examination in August and the second in November This decline coincided with reduced

physical training, and both groups appear to have been influenced to the same degree The result is in agreement with numerous observations, showing that a high physical working capacity obtained by training declines rapidly when the physical training is stopped The decline was so great for the cadets that, by the time the cruise started, their physical working capacity was quite low, about the same as for the conscripts

During the cruise (see Fig 1) the working capacity of the cadets who received extra training rose significantly The other two groups showed no significant improvement, although their capacity varied from month to month The changes in their capacity appear to have had a relation to the climatological changes Capacity was thus higher at lower air temperatures (below 20°), and lower at higher air temperatures (above 20°) It should be noted that the curves for Groups II and III follow each other fairly closely during the climatological changes This observation is in agreement with the results obtained in previous studies (1, 3, 5, 8, 12, 18) No such variation in working capacity, however, appeared in Group I (cadets with extra training) Since the working environment, including climatological conditions, was the same or similar for all three groups, this development of the working capacity of Group I is taken to be an effect of the additional training This too is in good agreement with previous results from short-term studies (25 26 32), in which it was found that physical working capacity was not affected so markedly by changes in the air temperature if physical training had raised the capacity to a relatively high level The present results suggests that this situation persists over longer periods of time, when the air temperature gradually rises or falls several times, producing considerable variations

The results also show that simple, rational training can maintain physical working capacity at a good level even within the confined space of a warship

During the cruise, hand grip strength underwent a significant increase both in the group receiving extra training and in the other two groups This improvement in performance, which may partly be a consequence of work and general training on board, does not appear to follow or influence the development of working capacity Nor does muscular strength appear to be markedly influenced by changes in temperature This suggests that physical training should definitely engage the circulatory organs if deteriorations in working capacity are to be limited or avoided

No significant change in blood pressure was observed in any of the groups during the cruise This could be due to the fact that the changes in air temperature occurred relatively slowly, and were not so pronounced as in certain short term studies in which changes in blood pressure have been observed (1, 3, 7)

Summary

Physical working capacity and muscular strength, plus a number of somatometric measurements, were followed in three experimental groups on board HMS

Älvsnabben during a 5 month cruise around South America 1957—58 One group received additional physical training on board and displayed a rising physical working capacity which was not influenced to any great extent by changes in climate This group lost most weight The other two groups, which received only the ordinary physical training, showed no improvement in working capacity during the cruise, but did display variations that seem to have been related to changes in the air temperature They lost very slightly in weight. Hand grip strength showed a significant increase in all three groups

Training that primarily engages muscular strength would seem to be incapable of producing any considerable effect on the fluctuations in physical working capacity that occur with marked changes in climate Training that engages the circulatory organs can, however keep physical working capacity at an satisfactory level Such training can be organised on board a ship

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From The Military Medical Examination Centre Karolinska Sjukhuset Stockholm 60

Application of a Maximal Exercise Test for Estimating the Physical working Capacity of Recruits for Compulsory Military Service

By

K. Linroth, B Nordgren, B Simonsson and G Tornvall

In the determination of the physical working capacity in connection with screening examinations it is usually necessary to rely on simpler and more rapid methods than is the case under laboratory conditions In the military forces, for instance, there is an urgent need for screening tests for assessing the working capacity Experience suggests that a maximal exercise test on the bicycle ergometer can be arranged so as to meet reasonable demands of both simplicity and accuracy (6) The object of the present study was to examine the application of such a test the principle of which involved measurement of the maximal time during which the subject could sustain a certain constant load In this way practical experience might be *obtained of the application of the method* under the special experimental conditions associated with the registration of military recruits An attempt was also made to establish the reproducibility of the method and to form an impression of the information that can be yielded by investigations of this type.

Material

The exercise test was included in the medical examination carried out in the autumn of 1963 on 917 recruits, mostly 18 years of age at registrations arranged in the recruiting areas of Örebro and Halmstad As it was impossible to make a completely random selection of participants the results presented here are not fully representative of the population in question Persons suffering from diseases where it was considered that maximal physical effort should be avoided were excluded from the tests. They numbered 13 six of them with injuries to the extremities two with residual paresis after poliomyelitis two with valvular heart disease one with suspected electrocardiographic changes (no check could be made), 1 with acute bronchitis and 1 who had recently had an operation for inguinal hernia.

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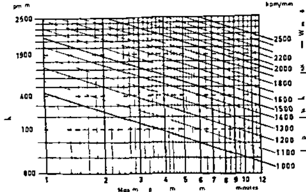
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F g 2 Nomogram for est ma tion of maximal working capa city (W_{max6}) from experimentally obtained load t me values

group examined at the Military Medical Examination Centre The relationship between W_{max6} and the maximal dura tion of exercise is represented by the fol lowing expression

$$\log W_{max6} = \frac{\log T - \log 6}{\tan \alpha} \log N$$

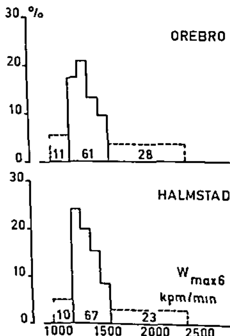
where

W_{max6} is the maximal load that the sub ject is calculated to be able to sustain for 6 minutes

N is the standard load (1400 kpm/min)

T is the maximal duration of exercise (minutes) at load N and α is the mean angle that the regression line makes with the axis of abscissa

In other contexts where the method has been applied the procedure has been repeated with different loads From one or two preliminary runs the load could be found for which the maximal dura tion of exercise was near 6 minutes This enabled a more accurate value of W_{max6} to be obtained for the individual subject (Tornvall unpublished result) In the present study however only one of the two days assigned to the medical exami nation was taken for the working capacity test with a few exceptions where both days were used in order to determine the reproducibility As the main object of



F g 3 Frequency distr butions of W_{max6} values recorded at registration in two groups of men liable for m litary service

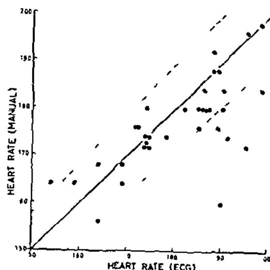


Fig 1 Final heart rates after maximal exercise Relation between values *recorded manually* by palpation of carotid artery and values calculated from Ecg Unbroken line denotes ideal agreement — broken lines *correspond to* ± 5 per cent deviation

Subjects who had been classified at the clinical examination as unfit for military service or assigned to a low grade were tested in the usual way — if there was no reason why the maximal exercise should not be performed

Method

The exercise test was performed with the subject seated on a bicycle ergometer with electrical breaking and automatic correction for variation in pedalling speed (the Elema bicycle ergometer as designed by Holmgren & Mattson (3)) In a small number of cases electrocardiograms were recorded during and after exercise (Mingograph B 42, Elema-Schonander AB, Solna) The subject was required to perform a standard exercise with a load of 1400 kpm/min, which was kept constant during the test No preliminary warming up at low rate was required The investigator encouraged the subject to continue the exercise as long as possible, up to 12 minutes Immediately afterwards the investigator took the pulse manually over the carotid artery for 15 seconds This procedure may incur an error due for instance to difficulty in finding the artery, in beginning the count just at the end of the exercise, and in counting rapid heart rates, for this reason a separate study was made on a group of 36 subjects from the Halmstad series of the agreement between the rates recorded manually and electrocardiographically

As is seen from Fig 1, with the manual count there was a tendency to underestimate the high rates, for the lower rates there was a closer agreement

In advance each participant was informed of the nature of the test and of the possibility to find whether the subject was exhausted by the exertion He was informed also that if he did not cooperate fully in the test he would be required to repeat it It was considered reasonable to assume that there was inadequate cooperation if the final heart rate was less than 150 (Fig 5) To avoid as far as possible any influence of irrelevant factors on the results the time was not announced during the course of the test The recorded duration was converted by means of a nomogram to the maximal work intensity that the subject was calculated to be capable of sustaining for 6 minutes — denoted by W_{max6} (Fig 2) The nomogram, which indicates the mean relationship between the load and the maximal duration in bicycle ergometry, was drawn on the basis of the results of earlier tests on a recruit

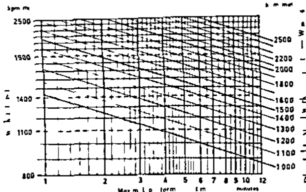


Fig 2 Nomogram for estimation of maximal working capacity (W_{max6}) from experimentally obtained load time values

group examined at the Military Medical Examination Centre The relationship between W_{max6} and the maximal duration of exercise is represented by the following expression

$$\log W_{max6} = \frac{\log T - \log 6}{\tan \alpha} \log N$$

where

W_{max6} is the maximal load that the subject is calculated to be able to sustain for 6 minutes

N is the standard load (1400 kpm/min) T is the maximal duration of exercise (minutes) at load N and α is the mean angle that the regression line makes with the axis of abscissa

In other contexts where the method has been applied the procedure has been repeated with different loads From one or two preliminary runs the load could be found for which the maximal duration of exercise was near 6 minutes This enabled a more accurate value of W_{max6} to be obtained for the individual subject (Tornvall unpublished result) In the present study however only one of the two days assigned to the medical examination was taken for the working capacity test with a few exceptions where both days were used in order to determine the reproducibility As the main object of

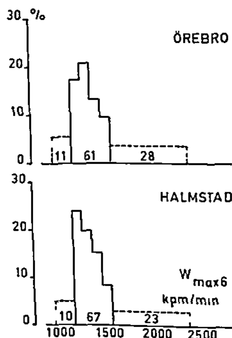


Fig 3 Frequency distributions of W_{max6} values, recorded at registration in two groups of men liable for military service

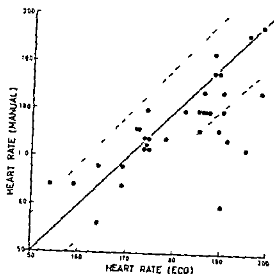


Fig 1 Final heart rates after maximal exercise Relation between values recorded manually by palpation of carotid artery and values calculated from Ecg Unbroken line denotes ideal agreement — broken lines correspond to ± 5 per cent deviation

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Table I Relationship between W_{max6} and some body dimensions

W_{max6} kpm min		<1150	1150—1250	1250—1350	1350—1450	1450—1550	>1550
Number		98	193	189	133	83	235
Weight (kg)	Mean	57.5	60 7	64 4	67 1	67 3	70 2
	SD	0 6	0 4	0 5	0 7	0 8	0 5
Height (cm)	Mean	174 6	176 4	177 1	177 7	178 1	180 0
	SD	0 6	0 4	0 5	0 6	0 6	0 4
Handgrip	Mean	58 5	60 9	64 0	65 5	65 5	67 4
force (kp)	SD	1 1	0 6	0 7	0 9	1 1	0 6
Width of	Mean	9 60	9 77	9 88	9 96	9 92	10 13
femoral condyle (cm)	SD	0 04	0 03	0 03	0 04	0 05	0 03
Heart	Mean	516	557	593	624	642	686
volume (ml)	SD	9	7	8	10	15	8

Note — The heart volume was calculated from fluorograms taken in the erect position The above values are on average slightly lower than those that have been reported for similar series where the heart volume has been calculated from radiographs in the supine position *The difference is* ascribable in some measure to orthostatic factors and probably to projection conditions and differences in the method of calculation

[a] kp kilopond

in accordance with the requirements of the military examination Incidentally no account of the results of the working test was taken in deciding military classification (Fig 4)

As would be expected assignment to a low fitness group implied a tendency for selection of persons with low physical working capacity

In the 17 subjects in low grades recording more than 1500 kpm/min the reasons for the lower classification were as follows mental disorder and defective *vision in 4 each* arterial hypertension allergy and foot anomalies in 3 each

The results of an examination of the relationship between the maximal working capacity and various body dimensions are presented in Table I

No calculation was made of the correlation between various parameters But from the means for the various body dimensions for the groups with different working capacities it is seen that W_{max6} was related not only to height, weight and heart volume but also to the muscular power and build

To determine the final heart rate for the individual subject it was necessary to find the normal range

As shown earlier the heart rate is dependent on the duration of exercise (6) so that on average the final heart rate is not the same for short as long periods of maximal work The relationship between the duration and the heart rate after maximal exertion was therefore examined (Fig 5) This study included only subjects who had been classed as completely fit (groups 1 and 2)

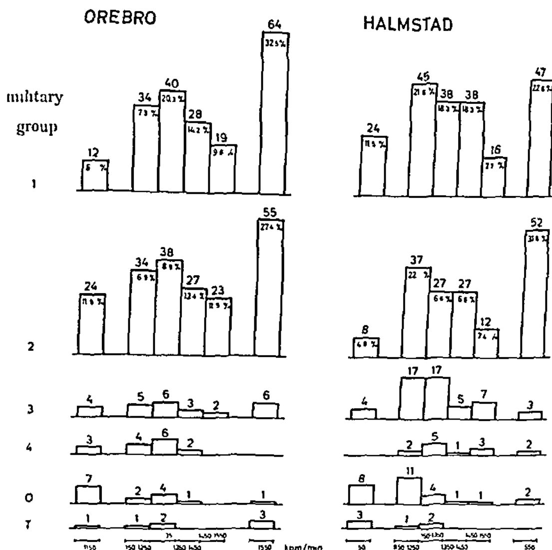


Fig 4 Graphical presentation of two series of recruits divided into sub-groups according to *military fitness group* and to recorded value of maximal working capacity (W_{max6})

the test was to examine the requirements for grouping the recruits according to working capacity only one standard load was used though this meant that for subjects at the extremes of the range the individual values were not determined to a high level of accuracy

Results

The values recorded for the working capacity are presented graphically in the following frequency diagrams for the Örebro and Halmstad series (Fig 3)

It is seen that about 60 per cent of the subjects attained a value of between 1150 and 1550 and about 25 per cent exceeded 1550 The working capacity was slightly higher on average for the Örebro series

As some of the subjects at the medical examination were assigned to low fitness grades for various reasons it was of interest to divide the series into sub-groups

incentive that may tend to lower the final heart rate are bone and joint diseases, respiratory illness, mental disorders and other conditions that may limit the maximal working capacity

Many studies have been carried out to determine the working capacity by means of the bicycle ergometer In one of them (21 subjects) the mean maximal heart rate was 204 beats a minute (SD = 7) with a corresponding simultaneous lactic acid concentration of 107 mg/100 ml (2) In 123 recruits during service Nordesjo obtained by the method designed by Tornvall (6) a mean final heart rate of 188 (SD = 10) and a lactic acid concentration of 110 mg/100 ml It would thus seem that there is a difference in the maximal heart rate whereas the requisite anaerobic metabolism would seem to be in agreement

The fact that the final heart rate was lower for short durations of maximal exercise suggests that a period of adjustment is required before the maximal heart rate can be recorded

The present values were also compared with those recorded earlier for another group of ordinary recruits during military service (6) If account is taken of the fact that a slightly lower heart rate is obtained when the pulse is taken manually especially at high rates the final rate would seem to be the same for the subjects liable for military service as for those examined earlier

As mentioned earlier the connection between the final heart rate after maximal work and the incentive would seem to provide a means of recognizing subjects that did not cooperate enough in the study It is then however necessary to eliminate other factors that might affect the final heart rate If in the individual case the final heart rate should be less than the mean recorded rate minus the standard deviation (for the same duration) a low level of incentive might be suspected In view also of the risk of an error in the determination of the heart rate by palpating the carotid pulse it would seem that a replicate determination should be performed in these cases

In the planning of the medical tests it was arranged that the exercise test should come last. Moreover there was always a physician in attendance where the test was carried out While no serious complications associated with the test were noticed mention might be made of one case After cycling at the standard load for 9 min 35 sec one of the Halmstad group complained of visual blackout The test was discontinued and the heart rate was then 112 About 45—60 sec after the load the electrocardiogram showed a sinus rhythm of about 150 and normal tracings No explanation of the blackout could be found

For further studies with the maximal exercise test it is important to establish norms for participants in the light of the experience gained in the present study It is evident that anyone that has or that has recently had an infection with a high temperature or any other general affection should avoid maximal physical effort However in practice — for instance in military service it is difficult to exclude from physical effort all persons who have or have recently had mild catarrhal infection A careful medical examination before the test and

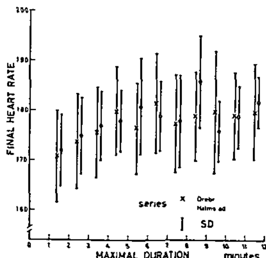


Fig 5 Mean final heart rate (manual) after maximal exertions of different duration in two series of recruits, classed as completely fit at registration for military service

The reason for including only completely fit subjects was the need to eliminate persons with disorders that might possibly affect heart reaction during exercise

The reproducibility of the method has been tested earlier in The Military Medical Examination Centre on a group of submarine volunteers The variability of the replicate determination was found to be 4 1 per cent for a single determination (6) As the experimental conditions in the present study were not comparable and the series consisted of different categories of subjects, an examination was made of the reproducibility under these particular conditions From the Halmstad series 51 men were selected who were required to perform the ergometer test on 2 consecutive days at a standard load of 1100 kpm/min until subjectively exhausted The variability was found to be 4 8 per cent, a value that is thus quite close to that obtained earlier

Discussion

In the method used for the exercise test, in which the performance is measured under certain standard conditions, the value is dependent on a number of factors, the most important of them for young healthy subjects probably being the circulatory and respiratory functional capacity, the muscular power and the incentive for the examination

The incentive may be assumed to vary not only from one person to another but also from one situation to another Among the factors that tend to increase the incentive are respect for instruction, personal influence of the investigator, reward for good performance and the competitive spirit Examples of factors that diminish the incentive are the reluctance to suffer the discomfort involved by maximal physical effort, and the apprehension about being selected for particularly strenuous duties if high values should be obtained for the selection tests [1]

On an earlier occasion at the Military Medical Examination Centre two military series with different incentives one of the series consisting of ordinary recruits and the other of volunteers for special duties were required to perform an exercise test It was found that the final heart rate after exhaustive work was on average higher for the group with the greater than with the lesser incentive

Among the other factors than lack of

[1] A group of recruits were required to perform the exercise test on 2 occasions (prolonged exercise) on the second occasion when a monetary reward proportional to the duration was offered the performance was strikingly better (Ahlborg unpublished result)

those with the lowest working capacity the upper limit can be set at 1250 kpm/min The proportions of the Örebro and Halmstad series falling in this class were 28 and 34 per cent, respectively

For more exact basis for such a classification it is necessary to await the opportunity to carry out studies in different parts of the country on series representative of each recruiting area

Summary

A method for finding the maximal working capacity by means of bicycle ergometry was tested on two groups of men liable for military service, totalling 917 persons The results were expressed as the maximal rate of work that the subject could sustain for 6 minutes

The frequency distribution for the two series is presented for all the subjects and for sub-groups according to military category For about 60 per cent of the subjects the maximal working capacity was between 1150 and 1550 kpm/min (for 6 min) and for about 25 per cent it was above 1550 a few individuals had values below 1150

At the end of the maximal exercise test the heart rate was recorded and normal values derived The possibility of using the final heart rate to identify subjects lacking in incentive is discussed

Replicate determinations showed that the reproducibility of the method was 4 8 per cent of the mean for the single determination

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then supervision is however, considered an adequate safeguard Nonetheless, a continuous monitoring electrocardioscopic check would be advisable so as to be able to observe pathological alteration without delay

As alternatives to the exercise test used, other methods might be applied — for instance the submaximal test worked out at the Karolinska Hospital with a gradual increase in loads (4) This would provide an objective measure (unaffected by incentive) which would be related to the circulatory capacity A sub-maximal test, however, is invariably attended by greater uncertainty in the calculation of parameters of maximal function even if account is taken of, for instance, the fall in the mean maximal heart rate with age In addition, the gradual increase in the load would make it easier to detect in time any rhythmic anomalies or other complications For older persons where medical factors assume prime importance this is the only approach

A simplified method for determining the physical working capacity by using a submaximal load has been used in the Royal Swedish Air Force (1) The heart rate was determined after 6 minutes at a load of 900 kpm/min on a bicycle ergometer From the established relationship between the heart rate at sub-maximal exercise under steady state conditions and the maximal oxygen uptake capacity (8) the corresponding value for this maximum can be extrapolated By this method it is also possible to find the working capacity, at least in classes, from the heart rate at 900 kpm/min The method involves an extrapolation for calculation of the maximal working capacity, but on the other hand it is rapid to carry out (6 minutes' cycling) Direct measurement of the maximal oxygen uptake (including measurement of the lactic acid concentration in the blood) during exercise (7) is too time consuming for screening examinations A comparison between these methods and others for measuring the physical working capacity in recruits is being carried on

For young persons in good health, as those in the present series, it would seem that there is no special risk attached to using the maximal exercise test. The chief advantages of this test, performed as described, are its simplicity and rapidity

In the present study, as mentioned, the values of maximal working capacity were on average slightly higher for the Örebro than for the Halmstad series It is probable that this difference is due to the fact that a number of the recruits at Örebro, namely those at the higher secondary schools, who are presumably less fit on average, could not participate in the study on organization grounds

Although the series are thus not fully representative, the results would seem to allow of certain conclusions regarding the planning of future attempts of classification according to physical capacity For instance to select persons with particularly good physical working capacity the lower limit for W_{max6} may be put at about 1550 kpm/min

In the present study this elite group constituted 28 per cent of the Örebro series and 23 per cent of the Halmstad series On the other hand to separate

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bring about an increase of RBF, and, by means of this drug Werkö (15) prevented a decrease in the RBF in patients which was due to exercise. But since some investigators consider that sympathetic activity increases during vasodilatation induced by hydralazine (1), one group of animals was treated simultaneously with pentolinium and hydralazine

Materials and methods

In all the animals arterial blood pressure and changes in the pulse rate during the experiment have been recorded The plasma renin activity was determined in two samples of plasma, obtained from each animal in order to assess the animals ability to react to bleeding by an increase of plasma renin activity

Preparation and treatment of animals Adult female albino rats weighing between 200—240 g were used Before the drugs were given all the animals received the same pretreatment Rats were anesthetized by an intra peritoneal injection of Nembutal (6 mg per 100 g body weight) In the anesthetized animals first the right common carotid artery was cannulated and then connected to a blood pressure recording device (EMT 456 ELEMA Stockholm Sweden) Immediately after this a closed cannula was introduced into the left common carotid artery and was later used for bleeding

According to the treatment given the animals were divided into four groups The first group consisted of controls after cannulation these animals were injected with 0 5 ml of saline solution per 100 g body weight In the second group 3 mg of hydralazine were given per 100 g body weight In the third group the animals received 2 mg of pentolinium per 100 g body weight In the fourth group hydralazine and pentolinium were administered simultaneously, the same dosage being used as in the second and third groups All injections were given intraperitoneally, and the amount of injected fluid was the same in each case.

Time schedule of the experiments The first recording of blood pressure and pulse rate was made immediately after occlusion of both common carotid arteries and before injection of drugs or saline solution The writer used was a 500 xy Recorder (Electroinstruments Inc. San Diego California USA) By increasing the speed of the x-coordinate, a recording was obtained from which the pulse rate was calculated The second and the third recordings were made 15 and 30 minutes respectively after injection After the third recording the animals were bled The closed cannula was opened, and the blood allowed to run into a cold heparinized, calibrated tube From each animal 2 ml of blood per 100 g body weight were taken The bleeding time varied between 2—5 minutes depending on the blood pressure, which was continuously recorded during bleeding Next recordings were made 15 and 30 minutes respectively after bleeding Finally after the last recording a second sample of blood was obtained in the same way as in the first bleeding

Determination of plasma renin activity The plasma renin activity was determined in the first and second samples of

From the Department of Clinical Physiology, Karolinska Sjukhuset, Stockholm Sweden

Effect of Hydralazine and Pentolinium on Renin Release Caused by Bleeding in Rats

By

Lj Božović and J Castenfors

Few data in the literature support the view that the sympathetic system has a direct effect on renin release By direct effect is meant that sympathetic stimuli can cause renin independently of their action on blood vessels and renal hemodynamics Green (6) postulated that sympathetic overactivity may cause a release of pressor substances from the kidney Taquini et al (10) showed that three weeks after denervation rat kidneys contain less renin than the controls Infusion of catecholamines into the renal artery of anesthetized dogs is followed by an increase of the plasma-renin activity in renal venous blood (14) Even when a low dose of infused norepinephrine was given, which changed only very slightly renal functions, the renin release was significant Recently, a similar observation was reported (13), and moreover, the author pointed out the interesting fact, that the renin release, caused by catecholamine infusion or renal nerve stimulation, can be prevented of osmotic diuresis is induced

In the present study the influence of the sympathetic system on plasma renin activity has been investigated in rats treated with drugs which can modify sympathetic activity and renal blood flow (RBF) The experiments were performed on rats where both common carotid arteries were occluded a few minutes before starting the treatment Animals prepared in this way, but without the administration of drugs, will subsequently be referred to as controls Renin release has been caused by standardized bleeding, which is known to increase the renin activity of the peripheral blood (7) One group of animals was treated with pentolinium tartrate (Ansolysen, May and Baker) in order to decrease the sympathetic overactivity caused by the occlusion of both common carotid arteries and by bleeding As sympathetic overactivity gives rise to a decrease of RBF, a second group of animals was treated with 1 4 dihydrazinophthalazine metansulfon (Nepresolin, Ciba) Reubi (9) has shown that hydralazine can

TABLE I Effect of bleeding on the plasma renin activity in rats treated with hydralazine and pentolinium.

Treatment	Number	Renin activity in mμg/100 ml plasma			P
		Before bleeding	After bleeding	Mean diff ±SE*	
Control	8	1040	*2660*	*1620±460*	<0 02
Hydralazine	8	2620	4500	1880±410	<0 01
Pentolinium	6	*880*	*1230*	*350±480*	>0 1
Hydralazine and pentolinium	6	1710	1800	90±330	>0 1

* SE = Square root of the variance of the mean difference divided by the number of animals in the group

ditions The number of animals in each group is indicated in the figure

In groups where hydralazine or pentolinium was applied a decrease in blood pressure was recorded After 30 minutes the blood pressure in all the groups was relatively stabilized In the control animals bleeding caused a sharp drop in the blood pressure, and in the animals treated with the drugs a further decline was observed The fourth recording showed that immediately *after bleeding* the blood pressure in all the groups was within the same range The rise in the blood pressure during 30 minutes after bleeding was significant only in the control group It should be pointed out that the changes in the pulse pressures showed the same trend in all the groups

The pulse rate decreased after treatment in the three groups of animals that were given injections with drugs After bleeding the pulse rate increased significantly only in the group treated with hydralazine In groups treated either with pentolinium alone or with pentolinium *and hydralazine*, the pulse rate did not change after bleeding whereas in the control group it decreased

Plasma renin activity The results of the determination of the plasma renin activity are given in Table I In the statistical analysis of the results the cross-over trial design was applied i e the value of the plasma renin activity in the first *samples was taken as the control value* for the second sample obtained from the same animal

The renin activity in the rat plasma was higher under the experimental conditions than the activity in human plasma as detected by the method employed The control animals and those treated with hydralazine reacted to bleeding with a significant increase in the plasma renin activity The reaction of the animals treated with pentolinium showed only a slight insignificant increase, and the increase in the plasma renin activity which was observed in animals treated with hydralazine, was inhibited by simultaneous *treatment* with pentolinium

Though the mean values of the plasma renin activity differed in the first samples these differences were not significant

After bleeding there was a change in

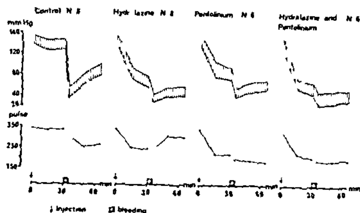


Fig 1

blood obtained from each animal Before centrifugation a small amount of blood was taken for hematocrit determination Blood was centrifuged in a cold room (+ 2° C) The interval between the bleeding and the transfer of blood to the cold room was less than five minutes From each blood sample one millilitre of plasma was taken and kept in a deep-freezer (—20°C) until the plasma renin activity was determined

The determination was made according to the method described by Boucher et al (2), with the following modifications Heparin, and not EDTA, was used as the anticoagulant Five millilitres of saline were added to the sample of 1 ml of plasma, and the diluted plasma was acidified for further processing as stated by Boucher et al The dry residue, which was obtained after evaporation, was dissolved in 1 ml of 20 volume per cent ethanol, and assayed by the blood pressure method on rats, which were 24 hours before nephrectomized The weight of these rats varied from 150 to 200 g They were anesthetized before the assay with sodium pentothal (6 mg per 100 g body weight intraperitoneally) and treated with pentolinium (2 mg per 100 g body weight given subcutaneously)

The 4-point assay design was used for the blood pressure responses to the intra venous injections of the standard and the extract obtained from plasma As standard angiotensin, Val^5-angiotensin II (Ciba) was injected in amounts of 2 5 and 5 nanograms The ethanol concen tration in the standard solution was the same as that in the extract. The extract was injected in amounts of 0 1 and 0 2 ml The values of plasma renin activity are expressed in nanograms of angioten-sin per 100 ml plasma (rounded off to 10 nanograms) The angiotensin refer-red to here is that which was formed during 3 hours incubation and subse quently extracted

Results

Blood pressure and pulse rate The data are summarized schematically in Fig 1 All points are arithmetic means of the data obtained from animals in one group, except the first point, which is the mean calculated from data on all the animals, because for all the groups these data were obtained before treat ment with drugs, i e under tre same con

TABLE I Effect of bleeding on the plasma renin activity in rats treated with hydralazine and pentolinium

Treatment	Number	Renin activity in mμg/100 ml plasma			
		Before bleeding	After bleeding	Mean diff ±SE*	P
Control	8	1040	2660	1620±460	<0.02
Hydralazine	8	2620	4500	1880±410	<0.01
Pentolinium	6	880	1230	350±480	>0.1
Hydralazine and pentolinium	6	1710	1800	90±330	>0.1

* SE = Square root of the variance of the mean difference divided by the number of animals in the group

ditions. The number of animals in each group is indicated in the figure.

In groups where hydralazine or pentolinium was applied, a decrease in blood pressure was recorded. After 30 minutes the blood pressure in all the groups was relatively stabilized. In the control animals bleeding caused a sharp drop in the blood pressure, and in the animals treated with the drugs a further decline was observed. The fourth recording showed that immediately after bleeding, the blood pressure in all the groups was within the same range. The rise in the blood pressure during 30 minutes after bleeding was significant only in the control group. It should be pointed out that the changes in the pulse pressures showed the same trend in all the groups.

The pulse rate decreased after treatment in the three groups of animals that were given injections with drugs. After bleeding the pulse rate increased significantly only in the group treated with hydralazine. In groups treated either with pentolinium alone or with pentolinium and hydralazine, the pulse rate did not change after bleeding, whereas in the control group it decreased.

Plasma renin activity. The results of the determination of the plasma renin activity are given in Table I. In the statistical analysis of the results the cross-over trial design was applied, i.e. the value of the plasma renin activity in the first samples was taken as the control value for the second sample obtained from the same animal.

The renin activity in the rat plasma was higher, under the experimental conditions, than the activity in human plasma as detected by the method employed. The control animals and those treated with hydralazine reacted to bleeding with a significant increase in the plasma renin activity. The reaction of the animals treated with pentolinium showed only a slight insignificant increase, and the increase in the plasma renin activity which was observed in animals treated with hydralazine, was inhibited by simultaneous treatment with pentolinium.

Though the mean values of the plasma renin activity differed in the first samples these differences were not significant.

After bleeding there was a change in

the hematocrit in the direction of hemodilution, with a mean of six per cent in all groups

Discussion

The elevated blood pressure, after occlusion of both common carotid arteries, can be considered as indirect evidence that before the treatment with drugs the sympathetic activity was increased in all the animals. Injections of drugs caused the expected drop in blood pressure.

The reaction of the control animals to bleeding resulted in lowered blood pressure and in a slowing of the pulse rate. This slowing of the pulse rate is possibly caused by hypoxia, especially when considered in relation to the ambient temperature. Bullard and Meyer (3) have shown that there is a connection between the hypoxic heart rate and the ambient temperature. At 10° C, rats react to hypoxia with a slowing of the pulse rate, and at 23°C, in some rats the pulse rate is decreased, in others accelerated. The experiments which have been described were made at room temperature, and the reaction of some of the control animals was an acceleration of the pulse rate but more often a decrease was observed. Animals treated with hydralazine reacted by an acceleration of the pulse rate and by the blood pressure being kept low during the 30 minutes following the bleeding. Both groups showed, after bleeding, some indirect evidence of an increase of sympathetic activity, in the controls there was vasoconstriction and in those who were treated with hydralazine, pulse frequency increased. In groups where the animals had been treated with pentolinium no changes in blood pressure or pulse rate occurred after bleeding, which can be considered as a sign of a rather complete sympathetic blockade.

Plasma renin activity, in the first samples before bleeding, showed marked individual variations. Perhaps the explanation of these variations is the short time between the treatment with drugs and bleeding. Hydralazine tends to increase, and pentolinium to decrease plasma renin activity under the conditions described. In normal rats without carotid artery occlusion plasma renin activity was about 500 ng angiotensin/100 ml plasma (unpublished observations). Both the control and the hydralazine treated animals were able to react to bleeding, but this did not apply to animals treated either with pentolinium alone or with pentolinium and hydralazine. This effect supports the view that the sympathetic system may play an important role in the mechanism of renin release.

The problem of what causes renin release under different conditions has not been solved (8). An assumption that has been once more or less generally accepted is that a decrease of the RBF can cause renin release (5). In the experiments described the RBF was not measured, but it can be assumed that in the control animals the RBF was decreased owing to sympathetic overactivity, particularly after bleeding (12). To judge by the blood pressures vasoconstriction was inhibited in the animals treated with drugs, but animals treated with hydralazine still reacted to bleeding with increase of plasma renin activity.

Changes in pulse pressure in the renal vessels have been postulated as cause of renin release (4) It has been pointed out that pulse pressure in the carotid artery did not differ essentially in the four groups of animals, but the data obtained do not enable us to say anything about the pulse pressure in the renal vessels According to Tobian's theory (11) a decrease in the stretch of the afferent arterioles is essential for renin release From the results obtained it can not be excluded that hydralazine and pentolinium acted differently on the stretch of the afferent aterioles Tobian also suggests the sympathetic system is *involved in renin release as an amplifier* of the signal which is acting on the juxtaglomerular cells Perhaps pentolinium abolishes this action of the sympathetic system and in this way prevents the release of renin caused by bleeding

In short current theories and the data obtained in our experiments do not enable us to say what causes the difference in reaction between animals treated with pentolinium and those not treated with this drug, but the direct action of the sympathetic system on juxtaglomerular cells cannot be excluded at least when renin release is caused by bleeding

Summary

The effect of a standardized bleeding (2 ml per 100 g body weight) on plasma renin activity has been studied in adult female rats weighing between 200—240 g Half an hour before bleeding the left common carotid artery was cannulated for recording blood pressures and pulse rates The right artery was also cannulated and used for bleeding This manipulation increased in animals the sympathetic activity These animals reacted to bleeding with a significant increase of plasma renin activity In the second group of animals pentolinium was injected, 30 minutes before bleeding to block the sympathetic overactivity, these animals did not react to bleeding by an increase of plasma renin activity In the third group hydralazine was applied to prevent a decrease in the renal blood flow caused by sympathetic overactivity These animals reacted to bleeding *with a significant increase of plasma* renin activity, which suggests that possibly, under these conditions, the decrease of RBF is not the cause of renin relase In animals treated simultaneously with hydralazine and pentolinium the rise in plasma renin activity caused by bleeding, is also inhibited On the basis of the results obtained, it is assumed that the sympathetic system may have a direct influence on renin release.

Acknowledgement

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From the Department of Clinical Physiology Karolinska Sjukhuset and the Institute of Hygiene, Karolinska Institutet, and the Department of Environmental Hygiene, National Institute of Public Health, Stockholm 60 Sweden

Renal Haemodynamics, Urine Flow and Urinary Protein Excretion during Exercise in Supine Position at Different Loads

By

JAN CASTENFORS and MAGNUS PISCATOR

A number of investigations (2, 6 7, 10, 23) have reported a decrease in renal plasma flow and glomerular filtration rate during exercise The most recent review is that of Wesson (22) The proteinuria associated with exercise has been recognized for a long time (15) The physiochemical nature of the exercise urinary proteins has been studied extensively (16 18, 19) but no simultaneous study of the renal haemodynamics and urinary protein excretion during exercise in man has been reported Furthermore few detailed studies on renal clearance and urine flow and their correlation to work intensity have been reported

The present investigation was therefore undertaken to study the urinary protein excretion the urine flow and the clearances of inulin and para aminohippuric acid during exercise at different loads and their correlation to heart rate used as index of work intensity The influence of hydration on renal functions during exercise was also studied Exercise was performed in the supine position to avoid postural proteinuria

Material

Eleven healthy male volunteers age 20—26 years, were investigated All were well informed about the procedure of the experiment Their work capacity was determined prior to the test and is defined as the amount of work the subjects could perform in the supine position on a bicylce ergometer at a heart rate of 170 beats per min (W_{170}), Sjostrand (20) Two subjects L H and I F were studied twice No ill effects were observed in any of the subjects Table I shows the age height, weight, W_{170}, individual work loads hydration and change in weight for each subject

Procedure

The experiments started at about 8 a m Water diuresis was induced by drinking 600 ml of water during the first 30

TABLE I Age, height, weight, W_{170}, work load, hydration and change in weight in all subjects

Subj	Code	Age	Height cm	Weight kg	W_{170}	Load Light work Kpm/min	% W_{170}	Load Heavy work Kpm/min	% W_{170}
1	RN	20	178	86	600	200	30	500	83
2	BR	23	179	74	1100	650	58	850	77
3	PS	22	186	73	950	400	42	600	63
4	LH[1]	22	183	76	1200	600	50	900	75
5	JP	26	185	69	1200	600	50	900	75
6	JF[1]	22	182	70	900	400	45	800	89
7	LL	23	182	71	1200	600	50	900	75
8	CH	23	174	75	1050	500	48	800	75
	Mean	22 6	181	74	924	494	47	781	77
1	KF	23	174	75	1050	600	57	800	76
2	KB	21	180	76	1150	600	52	900	86
1	LH[1]	22	183	76	1200	600	50	1200	100
2	JF[1]	22	182	70	900	400	44	1000	111
3	WZ	23	186	67	1000	500	50	1000	100

[1] some subjects
[2] values lost

minutes Hydration was maintained by drinking 150 ml of water every 20 minutes during the whole experiment Indwelling catheters (3) were introduced percutaneously into the brachial artery and the cubital vein of one arm A Foley catheter was inserted for collection of urine throughout the experiments The bladder was emptied by suprapublical pressure, rinsing with 30—50 ml of distilled water, followed by a 50 ml air wash After the priming dose of 8 mg para-aminohippuric acid (PAH) and 50 mg inulin per kilogram, a constant infusion of inulin and PAH at a rate of 0 5 ml/min was given through the catheter in the cubital vein The plasma concentration of PAH varied between 1 3—3 7 mg/100 ml The brachial artery catheter was connected to a pressure recorder, and in all but two experiments the blood pressure and heart rate were recorded in the middle of each period In two experiments in which blood pressure was not recorded, the pulse rate was calculated from ECG recordings Arterial blood samples were taken in the middle of each period After a 30 minute equilibration C_{PAH} and C_{IN} and other urinary parameters were determined for two 15 minute periods while the subject was at rest in supine position The exercise was performed in supine position, using an electrically braked bicycle ergometer (12) In the main group, which included 8 subjects

Hydration		
oral ml	I v 5 5 % glucose ml	Changes in weight kg
1650		−1 0
2250		−0 5
		−0 1
2400		−0 4
2250		*
2400		−1 0
2250		−0 6
2250		±0
2207		−0 5
1400	2000	+0 4
1800	2000	+1 1
2100	2000	*
1950	2000	+1 5
1950	2000	+0 1

the work was performed at a constant light work load for 45 minutes After one hour s rest the subjects exercised at a heavy work load for 45 minutes The individual work loads were chosen to obtain a heart rate of about 120 and 160 for the light and heavy loads respectively at the end of the 45 minute exercise The urinary parameters were measured during three 15 minute periods at each load and during three 20 minute periods in the hour between the two loads and again after the heavy load

In two subjects the procedure was identical with the exception that they were less hydrated during the light exercise period and that infusion of 2000 ml of 5 5 % glucose was started 30 minutes before the heavy exercise and was competed within one hour In three subjects exercise at the light work load was prolonged to 90 minutes (including six 15 minute periods) After one hour of rest, heavy exercise of 12 minutes duration (two 6 minute periods) was performed These subjects received 2000 ml of 5 5 % glucose before and during the heavy exercise in the same way as mentioned above

All but two subjects were weighed at the start and end of the experiment In 5 of the subjects urine was collected for protein studies from the night before and from the 12 hours after the exercise period

Methods

Para aminohippuric acid was determined by the method of Brun (5) *Inulin* was determined according to the method of Heyrovski (11) *Osmolality* in urine and plasma was determined by a modification of a method described by Bowman et al (4) The standard error of the method was for PAH ± 1 1 %, Inulin ± 0 7 % and osmolality ± 0 5 %
Protein concentration in urine was determined by the biuret method as outlined by Piscator (17) Methodological studies showed that inulin and PAH had no influence on the determination of urinary proteins

Electrophoresis of urinary proteins Urine was concentrated in collodion bags (Membranfiltergesellschaft Gottingen, Germany) under negative pressure The high urine flow and the bladder rinsing procedure during the experiment resulted in a low protein concentration in

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TABLE II Percentage change in the mean values caused by exercise The period of maximal mean change in relation to rest (Period II)

Parameter	Maximal change in relation to rest — Light work: Period	%	Heavy work: Period	%
Heart rate	V	+64	XI	+137
C_{PAH}	V	−32	XI	−53
C_{IN}	VII	−25	XI	−28
FF	V	+15	XI	+67
C_{osm}	V	−5	XIII	−49
C_{osm}/C_{IN}	VII	+35	XIV	−33
C_{H_2O}	VII	−62	XIII	−92
Urine flow	VII	−38	XIII	−83
U/P_{IN}	VII	+15	XII	+56
Ribonuclease excretion	XI	+23	X	+75
Protein excretion	V	+31	XI	+79

TABLE III Correlation between heart rate and different urinary parameters

Independent variable		Dependent variable: Parameter	Period[1]: Rest	Period[1]: Maximal change compared to rest		n	r	P
Heart rate		C_{PAH}	II	V	X	24	−0.89	<0.001
	Period[1]	C_{IN}	II	VII	XI	23	−0.62	<0.01
		FF	II	V	X	24	0.80	<0.001
Rest	II	Urine flow	III	VII	XIII	23	−0.92	<0.001
Light work	IV	C_{osm}	III	V	XIII	24	−0.59	<0.01
Heavy work	X	C_{H_2O}	III	VII	XIII	23	−0.88	<0.001
		U/P_{IN}	III	VII	XII	23	0.68	<0.01
		Protein	II	V	XI	24	0.38	>0.05
		Ribonuclease	II	VI	X	24	0.36	>0.05

[1] Periods used in the correlation

period of exercise C_{PAH} correlated well to the heart rate (Table III) In 3 subjects prolongation of the light load exercise period to 90 minutes (Fig 2), caused no further decrease in C_{PAH} Short severe exercise caused a *marked* decrease in C_{PAH}, the mean *decrease* being 72 % (fig 2) The C_{PAH} *returned almost* to the pre-exercise level within about 60 minutes *both after light and*

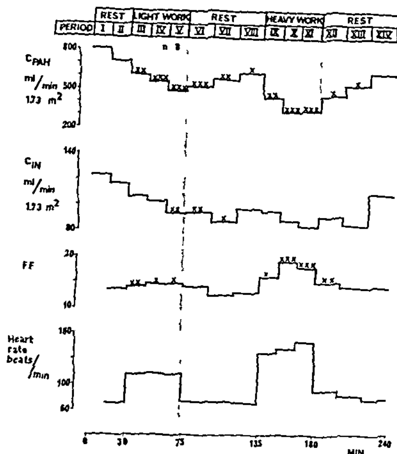


Fig 1 Effect of exercise at a light and a heavy work load on C_{PAH}, C_{IN}, FF and heart rate Mean values of 8 subjects are given In periods III—VIII, X represents the statistical difference (see methods) compared to period II and in periods IX—XIV, the statistical difference compared to period VIII (* $P<0.05$, ** $P<0.01$, *** $P<0.001$)

urine Because of this, and the difficulty of getting concentrates free from inulin, the urinary proteins from all periods could be studied by electrophoresis in only two subjects with relatively high protein excretion Electrophoresis was run in the LKB apparatus 3276 B at pH 8 6, barbital (Veronal) buffer, ionic strength 0 07, with separation time 16 hours and a current of 1 MA per strip Proteins were stained with Amido Black 10 B, and for quantitative evaluation a scanner was used *Ribonuclease* was determined by a turbidometric method (13) The standard was Bovine ribonuclease

Statistical analyses Current statistical methods were used The significance of individual differences between control periods (period II and VIII) and other periods was tested with Student *t* test The significance was expressed with the following symbols * $P<0.05$, ** $P<0.01$, *** $P<0.001$

Results

A *Renal haemodynamics*

Para-aminohippuric-acid clearance (C_{PAH})

There was a statistically significant decrease in C_{PAH} during light and heavy exercise compared to C_{PAH} prior to exercise (Fig 1) The maximal mean decrease compared to period II was for light exercise 32 % and for heavy exercise 53 % (Table II) In the last

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Heart rate	X	+64	XI	+137
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C_{IN}	VII	−25	XI	− 28
FF	X	+15	XI	+ 67
C_{osm}	X	− 5	XIII	− 49
C_{osm}/C_{IN}	VII	+35	XIV	− 33
CH O	VII	−62	XIII	− 92
Urine flow	VII	−38	XIII	− 83
U/P_{IN}	VII	+15	XII	+ 56
Ribonuclease excretion	XI	+23	X	+ 75
Protein excretion	V	+31	XI	+ 79

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		U/P_{IN}	III	VII	XII	23	0 68	<0 01
		Protein	II	V	XI	24	0 38	>0 05
		Ribonuclease	II	VI	X	24	0 36	>0 05

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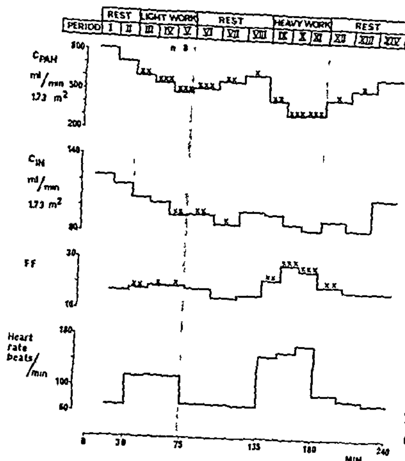


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Fig 3 Effect of exercise at a light a heavy work load on C_{PAH} C_{IN} urine flow (V) CH_2O and heart rate Mean values of 2 subjects are given Before and during the heavy exercise 2000 ml 5.5 glucose was infused intravenously

exercise (Fig 4) In the following periods during and after light exercise urine flow and C_{H_2O} decreased but these changes were not statistically significant compared to period II U/P_{IN} increased slightly after the light exercise period Heavy exercise was associated with a significant decrease in urine flow and C_{H_2O} and an increase in U/P_{IN} most marked in the periods of rest after exercise The changes in urine flow C_{H_2O} and U/P_{IN} correlated well with heart rate (Table III) Hydration did not inhibit the decrease in urine flow or C_{H_2O} or the increase in U/P_{IN} but caused a rapid increase in urine flow and C_{H_2O} and decrease in U/P_{IN} after exercise (Fig 5)

Osmolal clearance (C_{osm}) (C_{osm}/C_{IN}) *and plasma osmolity* (P_{osm})

Light exercise was associated with a tendency to decreased C_{osm} and increased C_{osm}/C_{IN} (Fig 4) Heavy exercise was associated with a significant decrease both in C_{osm} and C_{osm}/C_{IN} No significant increase occurred within 60 minutes after exercise P_{osm} showed a

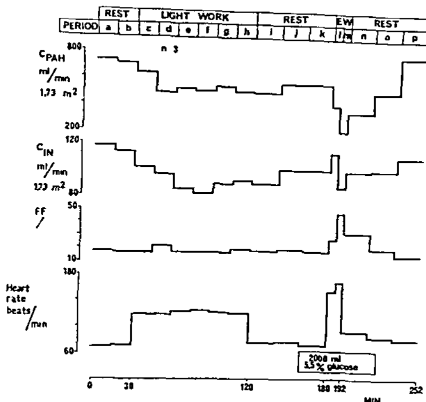


Fig 2 Effect of prolonged light exercise and short severe exercise (EW) on C_{PAH}, C_{IN}, FF and heart rate. Mean values of 3 subjects are given. Before, during and after the severe exercise 2000 ml 5.5 % glucose was infused intravenously

heavy exercise. Hydration insignificantly minimized the decrease of C_{PAH} during exercise (Fig 2 and 3)

Inulin clearance (C_{IN})

There was no statistically significant decrease in C_{IN} until the last period of light exercise (Fig 1). The maximal decrease occurred in the second period after exercise, 25 % (Table II). Heavy exercise did not further decrease C_{IN}. The decrease in C_{IN} was, however, correlated to heart rate (Table III). Prolongation of light exercise to 90 minutes (Fig 2) caused no further decrease in C_{IN}. There was actually a tendency to increase. Short severe exercise did not decrease C_{IN} (Fig 2). Hydration minimized the effect of exercise on the C_{IN} (Fig 2 and 3)

Filtration fraction (FF)

The FF was increased slightly during light exercise and more markedly so during heavy exercise (Fig 1). The FF was well correlated to heart rate (Table III). Prolongation of light load exercise did not chage FF, but short severe exercise caused a marked increase in FF (Fig 2)

B Renal handling of water

Urine flow, free water clearance (C_{H_2O}) *and urine/plasma inulin ratio* U/P_{IN}

Urine flow and C_{H_2O} were significantly increased in the first period of light

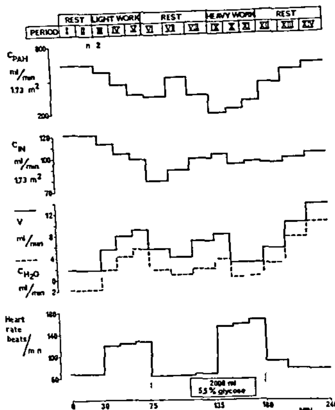


Fig 3 Effect of exercise at a light a heavy work load on C_{PAH} C_{IN} urine flow (V) CH_2O and heart rate Mean values of 2 subjects are given Before and during the heavy exercise 2000 ml 5 5 glucose was infused intravenously

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Osmolal clearance (C_{osm}) (C_{osm} C_{IN}) *and plasma osmolity* (P_{osm})

Light exercise was associated with a tendency to decreased C_{osm} and increased C_{osm}.C_{IN} (Fig 4) Heavy exercise was associated with a significant decrease both in C_{osm} and C_{osm} C_{IN} No significant increase occurred within 60 minutes after exercise P_{osm} showed a

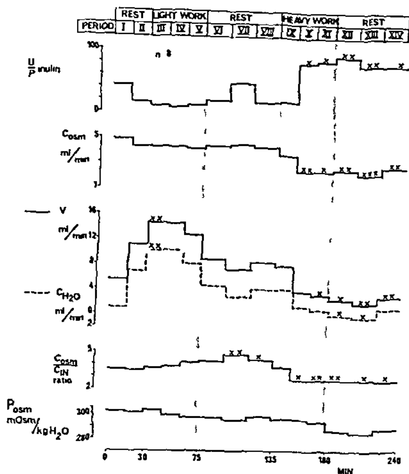


Fig 4 Effect of exercise at a light and a heavy work load on urine/plasma inulin ratio C_{osm} urine flow (V), CH_2O, C_{osm}/C_{IN} ratio and plasma osmolality (P_{osm}) Mean values of 8 subjects are given For further information see Fig 1

tendency to decrease successively during the whole experiment (Fig 4)

C *Urinary protein excretion*

Protein excretion (Fig 6)

The excretion of urinary proteins increased during exercise Light work caused a slight but not significant increase, maximal + 31 % in period V (Table II) Heavy exercise caused a more marked increase, which was statistically significant in the last two periods of work, mean + 79 % (Table II) Protein excretion did not return to pre-exercise level within 60 minutes after heavy exercise, as it did after light exercise Protein excretion showed a low correlation coefficient with heart rate (Table III)

Ribonuclease excretion (Fig 6)

Ribonuclease excretion was not significantly changed during the exercise periods In the third period of rest after light exercise, however, there was a significant increase compared with rest and, in the second period of rest after heavy exercise, a significant decrease in

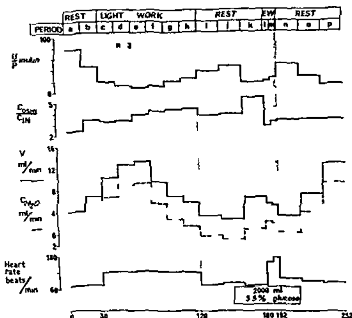


Fig 5 Effect of prolonged light exercise and short severe exercise (EW) on urine/plasma inulin ratio C_{osm}/C_{IN} ratio urine flow (V) C_{H_2O} and heart rate Mean values of 3 subjects are given Before during and after the severe exercise 2000 ml 5.5% glucose was infused intravenously

Fig 6 Effect of exercise at a light and a heavy work load on the urinary excretion of protein and ribonuclease Mean values of 8 subjects are given For further information see fig 1

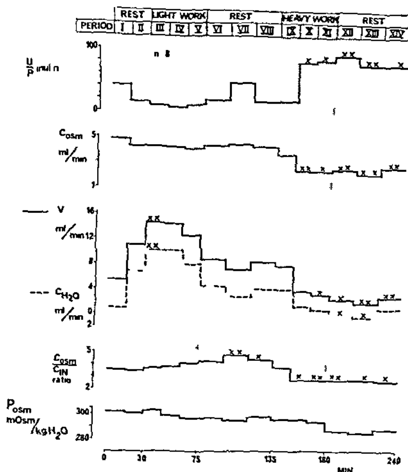


Fig 4 Effect of exercise at a light and a heavy work load on urine/plasma inulin ratio C_{osm} urine flow (V) C_{H_2O} C_{osm}/C_{IN} ratio and plasma osmolality (P_{osm}) Mean values of 8 subjects are given For further information see Fig 1

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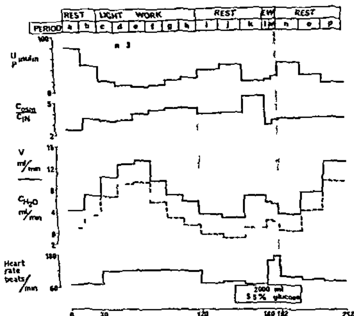


Fig 5 Effect of prolonged light exercise and short severe exercise (EW) on urine/plasma inulin ratio C_{osm}/C_{IN} ratio urine flow (V) C_{H_2O} and heart rate Mean values of 3 subjects are given Before during and after the severe exercise *2000 ml 5 5 % glucose was infused intravenously*

Before test
REST
LIGHT WORK
REST
HEAVY WORK
REST
After test
PERIOD
I II III IV V VI VII VIII IX X XI XII XIII XIV
PROTEIN µg/min
n 5
n 8
n 5
RIBO-NUCLEASE µg/min
Heart rate beats/min
0 7.8 HOURS
0 30 240 MINUTES
0 2,7 13,0 HOURS

Fig 6 Effect of exercise at a light and a heavy work load on the urinary excretion of protein and ribonuclease Mean values of 8 subjects are given For further information see fig 1

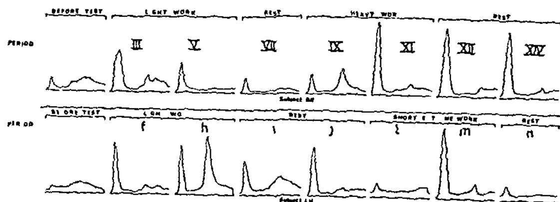


Fig 7 Scanned electrophoretic pattern at pH 8 6 in 2 subjects B R performed 45 minutes exercise at a light and a heavy load L H performed 90 minutes exercise at a light load and 12 minutes exercise at a very heavy load

ribonuclease excretion compared with rest before hevy exercise

Electrophoretical pattern (Fig 7)

Electrophoretical pattern of two subjects are shown in Fig 7 One subject (B R) shows an increased albumin fraction in the electrophoresis both during light and heavy work After light exercise the large albumin fraction disappears, but after heavy exercise the albumin fraction remains Subject L H shows also an increased albumin fraction during light exercise and extreme exercise of short duration, but in period h (light exercise) an increased β fraction was also observed

Discussion

Several practical problems must be considered when trying to evaluate renal function during exercise The methodological error of clearance determinations is large during low urine flow, and artificially increased hydration was necessary in almost all studies to keep this error within reasonable limits When evaluating the correlation between work intensity and renal function the degree of hydration has to be kept in mind The duration of exercise as well as the intensity are also integrated in the final change of renal haemodynamics during exercise In the present study an attempt was made to evaluate the influence of these factors

Several investigators have reported that renal plasma flow decreases during exercise (2, 6, 7 10, 23) Renal plasma flow has been found to decrease more markedly at a heavy than at a moderate load Investigations have been made with various degrees of hydration and exercise at different work loads has been performed mostly in separate studies and by different individuals In the present study with single subjects exercising at two different work loads heavy work decreased C_{PAH} with 54 % and light work with 32 % In the third period of exercise C_{PAH} showed a high correlation with heart rate (r = 0 89) which agrees closely with the results of Grimby (10) After exercise C_{PAH} returned almost to pre-exercise level within about 40 minutes both after

moderate and heavy work. Prolongation of the moderate work did not further decrease C_{PAH} which agrees with the findings of Chapman et al. (7). The intensive exercise until exhaustion decreased C_{PAH} to 75% of the resting value, which has also been reported by White and Rolf (23). Heavy hydration only slightly minimized the effect of exercise on C_{PAH}, which has probably contributed to the good agreement between the changes of renal plasma flow in this and previous studies.

The effect of exercise on the glomerular filtration rate has been studied by several investigators with contradictory results. Some report no change (1, 14), but most reports indicate a moderate decrease in inulin clearance or creatinine clearance (2, 6, 9, 10). In the present study there was a significant decrease in C_{IN} during light exercise lasting 1 hour (Fig. 1). The heavy exercise caused no further decrease in C_{IN}. By increased hydration the decrease in inulin clearance was almost inhibited during heavy exercise. During light exercise with limited hydration the decrease in inulin clearance was more marked (Fig. 3). Even during short intensive work until exhaustion the decrease in inulin clearance was almost inhibited by heavy hydration (Fig. 2). This suggests that variations in the degree of hydration has an important influence on C_{IN} during exercise and may partly explain the different results in earlier studies of C_{IN} during exercise.

The filtration fraction during muscular exercise is reported to increase by most authors with the exception of Barclay et al. (2) who reported a decrease of filtration fraction during exercise. In the present study the filtration fraction significantly increased both during heavy and light work (Fig. 1). The marked increase in filtration fraction during short severe exercise (Fig. 2) is probably due partly to the increased hydration.

A decrease in urine flow during exercise is observed in almost all studies. No correlation between urine flow and work intensity has been reported, and most studies indicate an increased urine flow a few minutes after exercise (22). The decreased urine flow may be the result of a decrease in glomerualar filtration rate or be due to liberation of antidiuretic hormone (ADH). The exact contribution of these two factors is difficult to evaluate. In the present investigations the subjects were heavily and as uniformly hydrated as possible. Light exercise caused only a slight and not significant decrease in urine flow and C_{H_2O}. The increase of urine flow and C_{H_2O} in the first period may be explained as the results of the initial hydration. The exercise period was started too early before urine flow rate was constant. Heavy exercise significantly decreased urine flow, simultaneously with only a slight further decrease in C_{IN}. The close relationship between urine flow and C_{H_2O} (Fig. 4) suggests that the main reason for antidiuresis during exercise was increased water reabsorption, presumably an ADH effect. Decrease in glomerualar filtration rate may have contributed to the decrease in urine flow during and after light exercise.

Plasma osmolality showed a tendency to decrease during the whole experiment in spite of the signs of ADH release. It

seems as if the ADH releasing effect of exercise is more potent than the opposing stimulus acting on the osmoreceptor in the hypothalamus The decreased plasma volume during exercise may stimulate ADH release, but heavy hydration with 5 5 % glucose did not inhibit the ADH release Presumably muscular exercise by reflex activation of the supraoptic nucleus, through its abundant connections with the hypothalamus and higher centres of the brain, stimulates ADH release (21)

The tendency to decrease in osmolal clearance during light exercise is caused by the decrease in C_{IN}, because C_{osm} C_{IN} remained virtually unchanged During heavy exercise tubular factors also participate in the decreased osmolal clearance, indicated by the significant lowering of the C_{osm} C_{IN} ratio Decreases are reported in the excretion of sodium (6) and urea (8) during exercise The lowered C_{osm}/C_{IN} ratio during heavy exercise suggests an increased tubular reabsorption of these substances

The increased urinary protein excretion has been attributed to many factors such as acidosis, mechanical trauma and renal vasoconstriction The similarity between exercise proteinuria and catecholamine induced proteinuria has fo cused attention on renal vasoconstriction as a cause of exercise proteinuria (18, 24) Most authors have reported the most marked protein excretion in the first post-exercise portion of urine, and Poortmans suggests that this is a washing-out phenomenon, secondary to the increased *urine flow after* exercise, which overwhelms the tubular reabsorptive capacity for proteins

The high urine flow rates in this study *seem to have augmented* the protein excretion, indicated by the increased urinary protein excretion in the two pre-exercise periods compared with the protein excretion the night before the test (Fig 6) This may partly be due to washing-out of uromucoids from the urinary tract

However, the most marked protein excretion occurred during exercise, simultaneously with decreasing urine flow, and returned towards pre-exercise level in the first post exercise period Urine flow and C_{IN} did not increase until 40 minutes after heavy exercise, which speaks strongly against a post exercise washing-out phenomenon in this study It cannot be excluded that the urine collected during the post exercise period in most earlier studies at low urine flow rates may have been mainly formed during and not after the exercise The excretion of the low molecular weight protein ribonuclease, which can be expected to be almost completely filtrable in the glomeruli and therefore primary, depending on changes in tubular reabsorption, showed no significant changes during exericse This suggests indirectly that changes in tubular reabsorption are of minor importance for exercise proteinuria

The increased filtration fraction during exercise may be the result of an increased glomerular filtration pressure secondary to an unchanged glomerualar filtration rate in spite of the decrease in renal plasma flow This suggests that increased glomerular filtration pressure in the glomerular capillaries, causing an increased filtration of plasma proteins

across the glomerular membrane during exercise, is of importance for exercise proteinuria

After heavy exercise the protein excretion did not decrease to pre exercise level within 60 minutes, but the renal haemodynamics were almost normalized One subject (B R), in whom electrophoresis of the urinary proteins was also studied (Fig 7) showed in the last period 40 minutes after exercise, the same large albumin fraction in the electrophoretical pattern as during exercise This may indicate that in addition to renal haemodynamic changes, exercise also causes an increased permeability of the glomerular membrane and that this effect lasts longer than the renal vasoconstriction Subject L H (Fig 7) also shows an increase in albumin fraction during exercise, but an increase in β fraction in one period during light exercise probably represents haemoglobin. This suggests that minor capillary bleedings in the bladder caused by the Foley catheter may in some period influence the determination of protein by the biuret method

Urinary protein excretion increased more at the heavy than at the light load which indicates that work intensity influences protein excretion This is probably mainly due to the different degrees of renal vasoconstriction

Summary

Clearance of inulin (C_{IN}) and para-aminohippuric acid (C_{PAH}) free water clearance (C_{H_2O}) and urinary protein excretion were measured in 11 healthy male subjects at rest and during supine exercise on a bicycle ergometer Work loads were between 400 and 1200 kpm/min and duration of the exercise between 6 and 90 minutes C_{PAH} decreased during exercise and was negatively correlated with work intensity (heart rate), ($r = -0.89$, $P < 0.001$) C_{IN} decreased during light exercise, but showed no further decrease during heavy exercise The findings suggest *that the effect of exercise on C_{IN} was* influenced by the degree of hydration The filtration fraction was increased during exercise Urine flow and C_{H_2O} showed a parallel decrease indicating release of antidiuretic hormone (ADH) Urinary protein excretion increased during exercise and decreased immediately after exercise It seems that renal vasoconstriction and increase in glomerular permeability are important factors in the mechanism of exercise proteinuria

Acknowledgement

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From the Departments of Internal Medicine (Head H Lagerlof M D) Clinical Physiology (Head T Sjostrand M D) and Surgery (Head J Adams-Ray M D) Karolinska Sjukhuset and King Gustaf Vth Research Institute (Head G Bake, M D) Stockholm Sweden

Effect of Exercise on the Elimination of Exogenous Triglycerides from Blood in Man

By

L. A Carlson, L. G Ekelund, S O Froberg and D Hallberg

Exercise acutely affects the metabolism of the free fatty acids of plasma (4) as well as the metabolism of endogenous[1] plasma triglycerides (6) Relatively little is known about the effects of exercise on the metabolism of exogenous[1] plasma triglycerides

In man exercise has been shown to decrease the turbidity of alimentary lipemia (8) and to reduce the increase in the concentration of plasma triglycerides (TG) normally seen after a fatty meal (18) It is not known however if these unanimous results are due to an accelerated removal of chylomicrones from blood during exercise or to a decreased influx of chylomicrones into blood caused by reduced fat absorption or orther factors occurring during exercise

We have recently described a new kinetic principle for the elimination of exogenous TG from the blood stream when injected intravenously either as an artificial fat emulsion or as chylomicrones isolated from the thoracic duct (5, 11, 14) According to this principle the exogenous TG are eliminated with a *maximal removal rate* (K_1), i e with a constant amount per unit time, above a certain plasma concentration and below this concentration there is a *fractional removal rate* (K_2), i e. the elimination is directly proportional to the TG level (5) It is thus possible to express the rate of removal of exogenous lipids from blood in mathematical terms

We have studied the effect of exercise on the removal rate of exogenous TG from blood by injection of a fat emulsion before, during and after exercise in healthy men and quantitated the removal constants during these different conditions

[1] By *exogenous* lipids we mean either lipids in chylomicrones entering blood from the thoracic duct during absorption of dietary fat or lipids in artificial fat emulsion If these lipids reappear into plasma after once having left the blood stream they are called *endogenous* lipids Endogenous triglycerides may also be synthesized from carbohydrates and from free fatty acids

Material and methods

Ten healthy male volunteers with a mean age of 23 3 years (± 2 3[1]), mean height of 178 5 cm (± 4 3) and mean

[1] Standard deviation

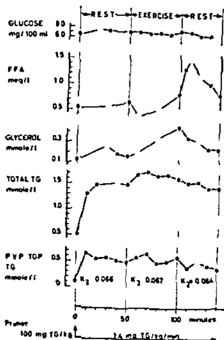


Fig 1 Effect of exercise on the fractional removal rate (K_2) of exogenous triglycerides (TG) from plasma in man Plasma levels of glucose, FFA and glycerol are also given A constant *infusion of fat emulsion* was given during the entire study The exogenous TG are recovered in plasma as PVP TOP TG

weight of 71 9 kg (± 13 5) were studied Some were studied twice with at least one month between the two studies The subjects were ordinarily trained with an average working capacity at a pulse rate of 170 beats/min of 1050 ± 113 kpm/min (21) The study was performed in the morning after an overnight fast A teflon catheter was introduced percutaneously into the brachial artery in one arm and another catheter into the cubital vein in the other arm No heparin was used After resting for about 30 min the experiment began with a constant infusion of fat emulsion in 4 studies and a single injection in 10 studies Between the single injections a slow infusion of 0 9 per cent saline was given through the venous catheter The subjects were first resting supine and then performed a supine work on an electrically braked cycle (pedal axis 22 cm above the table) They worked between 50 to 85 min at a constant load, mean 576 9 kpm/min (± 66 5) which was 55 8 % (± 5 9) of their working capacity at pulse rate 170, or 50 3 % of their maximal oxygen uptake After 10 min work the mean heart rate was 123 1 beats/min (± 6 0) and at the end of work 139 3 beats min (± 11 9)

The fat emulsion contained 20 per cent fat (Intralipid® 20 % (20), Vitrum, Stockholm) The constant infusion of the emulsion was started after rapid intravenous injection of a primer dose (0 1 g fat/kg) and the constant infusion rate was adjusted to correspond to 1 4—1 6 mg fat/kg/min The single injected dose was 0 1 g fat/kg when only K_2 was studied and 0 3—0 4 g/kg when K_1 and K_2 were studied

Arterial blood was drawn at intervals and the samples were collected into iced and heparinized tubes The tubes were centrifuged at slow speed (11) and plasma was collected for determination of FFA (23), glycerol (26) and exogenous TG Blood glucose was determined according to Marks (17)

The exogenous TG were isolated from plasma by fractionation with the PVP (polyvinylpyrrolidone) density gradient technique of Gordis (10) as modified (11) The top fraction of the PVP-gradient is considered to contain the exogenous TG (11) The TG content of the top fraction was analyzed according to Carlson (3)

Calculations In *the constant infusion* experiments the value for K_2 was cal-

culated from the mean value for the TG content of the top fraction during the periods as described previously (12) Before exercise a steady state level of TG in the top fraction had always been reached

In the *single injection* experiments the TG content of the top fraction was plotted against time in a linear as well as a semilogarithmic scale It was then decided visually which part of the curves that was linear and which was exponential The lowest value used for TG in the top fraction belonging to the arithmetically linear part was 0 5 mmole/ l The slopes were calculated as ordinary regression lines (method of least squares) (22) In two studies where it was doubtful if the elimination curve was linear or exponential the results were discarded

Results

Fractional removal rate (K_2) A typical experiment with constant infusion of emulsion is given in Fig 1 It is seen that the TG concentration in the PVP top fraction had reached a constant level before exercise This level was not changed when the subject started to exercise showing that $K_{_}$ was unaffected by exercise After exercise the concentration of exogenous TG tended to be somewhat lower indicating a slightly increased fractional removal after exercise

Fig 1 shows that exercise also during infusion of fat had the effects usually seen in the fasting state on blood glucose plasma FFA and glycerol Similar results with regard to these variables were obtained in studies 1 3 and 4

TABLE I Effect of exercise on the fractional removal rate K_2 (% per min) of exogenous triglycerides from plasma in man

Constant infusion

Study nr	Rest before	Exercise	Rest after	Δ Rb-E	Δ Ra E
1	3 9	3 4	4 4	+0 5	+1 0
2	6 6	6 7	8 4	-0 1	+1 7
3	10 5	8 9	11 5	+1 6	+2 6
4	9 5	10 0	10 5	-0 5	+0 5
M	7 6	7 3	8 7	+0 4	+1 5

Single injection

Study nr	Rest	Exercise	Δ Rb-E
5	9 6±0 8*	8 6±1 6	+1 0
6	5 8±0 2	7 1±0 4	-1 3
7	6 5±0 4	4.2±0 5	+ 2 3
M	7 3	6 6	+ 0 7

M = Mean value Rb = Rest before E = Exercise Ra = Rest after

* K_2 and its standard deviation was calculated according to Snedecor (22)

Table I shows that in the 4 subjects studied with the constant infusion technique exercise did not cause any significant change in the values for K_2 After exercise however there was an increase in K_{2} in all cases This increase was 1 5 ± 0 45 per cent per min (P = 0 05)

A study (nr 6) typical for the single injection technique is given in Fig 2 where the disappearance of the PVP top TG content is plotted semilogarithmically In this study K_2 increased slightly from 5 8 ± 0 2 at rest to 7 1 ± 0 4 per cent per min during exercise Table I shows that in the two other subjects

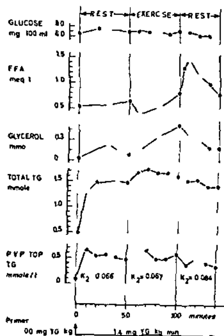


Fig. 1. Effect of exercise on the fractional removal rate (K_2) of exogenous triglycerides (TG) from plasma in man. Plasma levels of glucose, FFA and glycerol are also given. A constant infusion of fat emulsion was given during the entire study. The exogenous TG are recovered in plasma as PVP TOP TG.

weight of 71.9 kg (± 13.5) were studied. Some were studied twice with at least one month between the two studies. The subjects were ordinarily trained with an average working capacity at a pulse rate of 170 beats/min of 1050 ± 113 kpm/min (21). The study was performed in the morning after an overnight fast. A teflon catheter was introduced percutaneously into the brachial artery in one arm and another catheter into the cubital vein in the other arm. No heparin was used. After resting for about 30 min the experiment began with a constant infusion of fat emulsion in 4 studies and a single injection in 10 studies. Between the single injections a slow infusion of 0.9 per cent saline was given through the venous catheter. The subjects were first resting supine and then performed a supine work on an electrically braked cycle (pedal axis 22 cm above the table). They worked between 50 to 85 min at a constant load, mean 576.9 kpm/min (± 66.5) which was 55.8 % (± 5.9) of their working capacity at pulse rate 170, or 50.3 % of their maximal oxygen uptake. After 10 min work the mean heart rate was 123.1 beats/min (± 6.0) and at the end of work 139.3 beats/min (± 11.9).

The fat emulsion contained 20 per cent fat (Intralipid® 20 % (20), Vitrum, Stockholm). The constant infusion of the emulsion was started after rapid intravenous injection of a primer dose (0.1 g fat/kg) and the constant infusion rate was adjusted to correspond to 1.4—1.6 mg fat/kg/min. The single injected dose was 0.1 g fat/kg when only K_2 was studied and 0.3—0.4 g/kg when K_1 and K_2 were studied.

Arterial blood was drawn at intervals and the samples were collected into iced and heparinized tubes. The tubes were centrifuged at slow speed (11) and plasma was collected for determination of FFA (23), glycerol (26) and exogenous TG. Blood glucose was determined according to Marks (17).

The exogenous TG were isolated from plasma by fractionation with the PVP (polyvinylpyrrolidone) density gradient technique of Gordis (10) as modified (11). The top fraction of the PVP gradient is considered to contain the exogenous TG (11). The TG content of the top fraction was analyzed according to Carlson (3).

Calculations. In the constant infusion experiments the value for K_2 was cal

TABLE II Effect of exercise on the *maximal* removal rate (K_1) of exogenous triglycerides from plasma in man

K_1 (μmole TG/l plasma/min)

Study nr		Rest	Exercise	Δ Rest — Exercise
7		51±4*	45±3	6
8		46±3	43±5	3
9		50±7	60±7	—10
10		72±1	53±5	19
11		62±10	36±3	26
12	I	30±7	24±10	
	II	29±4	37±1	
	M	30	31	— 1
13	I	40±4	47±2	
	II	35±5	45±6	
	M	38	46	—8
M		50	45	5

* K_1 and its standard deviation was calculated according to Snedecor (22)

In cases 12 and 13 two injections (I and II) 30 minutes apart were given of the fat emulsion at rest as well as during exercise

M Mean value

Three subjects were studied on two different occasions 5 and 9 10 and 13 11 and 12

during exercise and at rest Table II summarizes the studies where values for K_1 were obtained A statistically sig*nificant effect* of K_1 *was obtained in* studies 10 and 11 where K_1 decreased during exercise. In the remaining 5 experiments 2 had smaller and 3 had greater values for K_1 during exercise than at rest but in none of these studies were the differences between the K_1-values statistically *significant* The average values for K_1 did, however not differ before and during exercise

In two cases K_1 was determined twice at rest and twice during exercise by repeated injections of the fat emulsion Table II shows the good reproducibility of the technique used for determination of K_1

Discussion

It is not known to what extent we are justified to draw conclusions from these studies with an artificial fat emulsion with regard to the effect of exercise on *the removal* of chylomicrones from the blood There are some observations, however, which suggest that the metabolism of the fat emulsion used and chylomicrones in rather similar at least with *regard to the elimination from* the blood stream The elimination of injected chylomicrones from blood has not only the same qualitative character but also the same quantitative kinetic constants as the emulsion (5, 14) Furthermore, human chylomicrones and thus emulsion have almost identical enzyme kinetic propert es when they as substrates are treated *in vitro* with postheparin clearing factor (1), an enzyme which probably is one of the rate determining factors in the removal of exogenous lipids from blood The appearance of recirculating endogenous plasma triglycerides has also very similar characteristics when either the fat emulsion or chylomicrones are injected (13 14) Evidence is also available showing that this fat emulsion is well utilized and tolerated It is thus possible to keep dogs in good health *and without weightloss for 10* weeks without food when this emulsion is given intravenously as one *ingredient in* complete parenteral nutrition (16) Thus there are many studies justifying the use

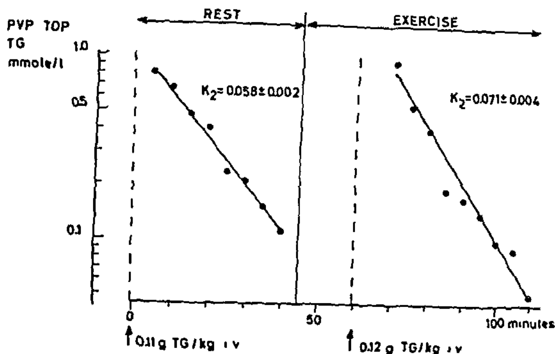


Fig 2 Effect of exercise on the fractional removal rate (K_2) of exogenous triglycerides (TG) from plasma in man A single injection of fat emulsion was given twice as indicated The exogenous TG are recovered in plasma as ' PVP TOP TG"

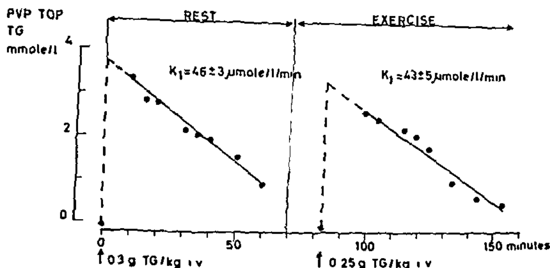


Fig 3 Effect of exercise on the maximal removal rate (K_1) of exogenous triglycerides (TG) from plasma in man A single injection of fat emulsion was given twice as indicated The exogenous TG are recovered in plasma as PVP TOP TG

studied with the single injection technique K_2 was insignificantly affected by exercise in one and was decreased in the other

Maximal removal rate (K_1) The linear plot in Fig 3 exemplifies the maximal removal rate at rest and during exercise in study 8 In this subject K_1 was similar

may be a decrease in the fat absorption or in the rate of formation of chylomicrones in the intestinal wall or in the rate of chyle formation In this connection it is of interest to point out that exercise is known to diminish the blood flow through the splanchnic vessels The decrease in alimentary lipemia may furthermore be due to unchanged influx and efflux of chylomicrones but a decrease in the recirculation of the fat e g in secondary particles and/or very low density lipoproteins as only turbidity or total TG content of plasma was estimated in earlier studies

Summary

The effect of exercise on the rate of removal of exogenous triglycerides (TG) from blood plasma was studied on 13 exercise experiments in 10 healthy male volunteers A fat emulsion which had previously been found to be removed from blood plasma in the same way as chylomicrones was given intravenously at rest and during exercise as a tracer for chylomicrones

The *maximal removal rate* of TG (K_1) decreased significantly in two studies and was statistically unchanged in the remaining five The *fractional removal rate* (K_2) decreased numerically in four and increased in three studies

These results cannot explain previous studies showing that exercise significantly reduces alimentary lipemia which is discussed

Acknowledgement

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of this artificial fat emulsion as a test substance for at least the early metabolism of chylomicrones

The volume of distribution of intravenously injected fat emulsion is considered to be equal with the plasma volume However, during exercise the plasma volume is rapidly reduced about 5 per cent (9,24) Therefore our calculated K_2-values with the constant infusion technique during exercise are probably to a small extent too high If *the plasma volume rapidly reaches* a new steady state volume during exercise the change in this volume should, however, not influence K_1 and K_2 as determined with the single injection technique

It has been shown from several laboratories (2, 15, 19) that isotopically labeled and intravenously injected chylomicrones are unevenly distributed in the body Some organs (e g liver) take up a lot whereas other organs (e g muscles) take up very little of the radioactivity This suggests that the elimination mechanism is unevenly distributed within the body

We consider that there are two main factors of importance for the process of elimination One is a structural factor(s) (e g enzymatic activity or pinocytosis) and the other the magnitude of the blood flow passing the active structure

The change from rest to exercise is known to cause significant changes in the distribution of blood between different organs (cf 25 7) During exercise the blood flow to the muscles increases while that to the liver kidneys and gastro intestinal tract decreases

When K_1 the maximal removal rate operates it is likely that the amount (activity) of the structural component, e g the amount of enzyme, is the rate limiting factor, and the magnitude of blood flow of less importance As we did not observe any consistent changes in the maximal removal capacity (K_1) it seems possible that the sum of structural activity was unchanged by exercise

When the removal rate is a first order reaction (K_2 level) it is likely that the magnitude of the blood flow is a major rate limiting factor If for instance the liver was the only organ for the removal one would anticipate that K_2 should decrease during exercise when liver blood flow decreases Our data suggest, especially if we consider the changes in plasma volume during exercise as discussed above, that exercise might lower K_2 in some persons The slight effect observed in combination with the sometimes unchanged or even increased K_2 values during exercise are, however, not easily compatible with the view that the liver is the only organ for removal of exogenous TG These effects on K_2 may on the other side be explained by a diminished blood flow through organ(s) with high removal activity counteracted by an increased blood flow through organs with low structural activity for the removal

The decreased alimentary lipemia observed during exercise in previous studies (8, 18) cannot be explained by the present results showing unchanged or decreased rate of removal of exogenous lipids from the blood If our results are valid also for chylomicrones there are certainly other mechanisms that can explain the reduction in alimentary lipemia by exercise There

From the Clinical Physiology Laboratory of Roslagstulls Sjukhus and IBM Medical Application Group Stockholm

Computerized Calculation of the Total Amount of Hemoglobin in the Body Using the Alveolar CO-Method

By

C CEDERLUND, A HOLMGREN, and B HÅKANSSON

This program calculates the total amount of hemoglobin in the body when using Sjostrand s alveolar CO method (appendix 1) (1–8) and the procedure developed at the Department of Clinica Physiology, Karolinska Sjukhuset Stockholm using the Stålex CO meter for the carbon monoxide analyses and the semiautomatic analyses described by Linderholm and Soderstrom (4) Patient information and experimental data are entered on a special form (appendix 2) then punched onto cards and processed by an IBM 7044 The results are printed out in list form (appendix 3) and arranged so that one part comprising the examination result can be copied for use on the wards while the entire list is filed for future reference

The normal values for total hemoglobin blood volume, hemoglobin concentration and initial carboxyhemoglobin concentration are obtained from linear regression equations (appendix 4) The examination results are compared with the normal values and evaluated by means of standard deviations (SD) for the normal equations

By programmed checks (appendix 4) we get supervision of the patient s preparation the patient s smoking habits leakage occurring during the measurement and non linearity of the measuring instruments

Input data

Input data are entered on a form which serves as a source document for punching Five cards are punched for each patient, and patients may undergo 1 2 or 3 examinations The first two cards (types 41 and 42) contain three cards (types 43, 44 and 45) contain examination information

Three data cards (types 41 42 and 43) must be present if data for a given patient is to be processed Identification of the patient is accomplished by means of a 6-digit number (see appendix 2) Each examination must be provided with its own 4-digit identification num

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ber Fields which are not filled on the form are printed out as —0 by the computer If a data field used for calculation is left blank, the corresponding calculation operation will yield an erroneous result

Program

The program is written in FORTRAN IV for the IBM 7044 (appendix 5) All information about a patient is read in and processed in accordance with the list of calculations presented in Appendix 4 The following checks are performed

a) *Sequence check* The cards shall be sorted by the first 8 columns (identification plus card type) in consecutive order If a card is out of sequence the program prints out the following error message your data cards are improperly sorted Identification No 000000 The run is therewith interrupted

b) *Identification check* The program checks that there is an identification card for every patient (card types 41 and 42) and at least one examination card (card type 43) If any of card types 41, 42, or 43 are missing, the program prints out the following error message *Card type 41, (42, 43) is missing for patient No 000000 Data for this patient are not processed and the program proceeds with the next patient*

A finish card indicating the end of the data cards shall be inserted after the cards of the last patient Nines shall be punched in columns 1 through 8 on the finish card (the test is for a 99 card type)

Output data

The result list (appendix 3) is arranged so that the left-hand part contains input data and the results obtained from a number of different steps in the calculations, while the right-hand part contains the result data Data for one patient are printed out on each page This together with the source information for punching and the punched cards is sent back to the hospital

The results are analyzed to determine their deviation from the calculated normal value, expressed in standard deviations (S D)

The evaluation represents the observed value minus the normal value (x) The following table gives the evaluation symbols and the descriptive evaluations

	Evaluation Symbol	Descriptive evaluation
x> 3SD	+***	Very high
3SD>x>2SD	+**	High
2SD>x> 1SD	+*	Somewhat above normal
1SD>x>−1SD		Normal
−1SD>x>−2SD	−*	Somewhat below normal
−2SD>x>−3SD	−**	Low
−3SD>x	−***	Very low

The list headings explain the result data, see Appendix 3

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Determination of the total amount of hemoglobin in the body using Sjostrand's alveolar CO method (1948)

Nomenclature and Symbols

S_{O_2} = O_2-saturation in blood
S_{CO_I} = CO saturation prior to administration of CO
$S_{CO_{II}}$ = CO-saturation after administration of CO
$P_{A_{O_2}}$ = Partial pressure of O_2 in alveolea
$P_{A_{CO}}$ = Partial pressure of CO in alveolae
M = 231 = empirical constant
$F_{O_{2_I}}$ = Fraction of O_2 in rebreathing system prior to administration of CO
$F_{O_{2_{II}}}$ = Fraction of O_2 in rebreathing system after administration of CO
F_A = Fraction of gas in alveolae
F_S = Fraction of gas in rebreathing bag
F_{CO_I} = Fraction of CO in rebreathing system prior to administration of CO
$F_{CO_{II}}$ = Fraction of CO in rebreathing system after administration of CO
P_B = Barometric pressure
K_{MY} = 0.95 = correction factor for the 5 % of CO combined with myoglobin and bone marrow
K_{STPD} = Factor for converting gas volumes to STPD
V_{inj} = Volume of CO administered to rebreathing system ATP
$F_{CO_{inj}}$ = Fraction of CO in administered gas, at present approx. 0.995
V_{app} = Volume of rebreathing apparatus, approx. 3 liters ATP
V_{RV} = Residual volume of patient, approx. 1.5 liters ATP
V_S = Volume in rebreathing bag after max. expiration, approx. 5 liters ATP
K_{P_B} = Correction for barometric pressure in the approximate equation
K_T = Correction for temperature in the approximate equation
[Hb] = Hemoglobin concentration in finger blood

Underlying principles

The in vitro affinity of carbon monoxide for hemoglobin is about 210 times greater than that of oxygen. Thus virtually all carbon monoxide administered to the body will combine with hemoglobin, a phenomenon that may be utilized to determine the total amount of hemoglobin, THb, in the body.

The principle involved here can be clarified by comparing it with oxygen transport in the blood. Let us assume that a blood sample has shown a given O_2 content and a given O_2-capacity. Their quotient is called the O_2-*saturation* of the blood. Since the same amount of CO combines with hemoglobin as O_2, namely 1.34 ml, the total amount of hemoglobin in the blood, THb, g, has a corresponding *total CO capacity* which can be expressed as

$$THb \cdot 1.34 \; (g \cdot ml \cdot g^{-1} = ml)$$

If V_{CO} ml STPD carbon monoxide is administered to this quantity of hemoglobin, the CO saturation of the total amount of hemoglobin will rise $\frac{\Delta S_{CO}}{100}$ which can be expressed as

$$\frac{V_{CO}}{THb \cdot 1.34} = \frac{ml}{g \cdot ml \cdot g^{-1}} = \frac{\Delta S_{CO}}{100} \tag{1}$$

CO is normally present in the blood due to

1 The formation of CO in conjunction with hemoglobin catabolism
2 Exposure to CO in daily life (smoking, automobile exhaust, etc)

As a result, S_{CO} must be determined as the difference between CO saturation before and after the administration of CO

$$\Delta S_{CO} = S_{CO II} - S_{CO I} \tag{2}$$

If equation (1) is rearranged as an expression of THb in which we substitute ΔS_{CO} from equation (2) we obtain

$$THb = \frac{V_{CO} \cdot 100}{1.34 \cdot (S_{CO II} - S_{CO I})} \tag{3}$$

The affinity of carbon monoxide for myoglobin is also very high however, and it has been estimated that about 5 % of the absorbed CO is actually combined with myoglobin in the muscle cells This is compensated for by multiplying THb with the factor K_{my} which for the present has been assigned a value of 0.95 Equation (3) thus becomes

$$THb = \frac{V_{CO} \cdot 100 \cdot K_{my}}{1.34 \cdot (S_{CO II} - S_{CO I})} \tag{4}$$

In order to calculate THb the absorbed CO and the CO saturation before and after the administration of CO must be determined

Determination of the CO saturation, S_{CO} *of the hemoglobin in the body*

According to Haldane the following equation for the relationship between partial pressure (P) and saturation (S) of O_2 and CO in whole blood applies at equilibrium in vitro

$$\frac{S_{CO}}{S_{O}} = M \cdot \frac{P_{CO}}{P_{O_2}} \tag{5}$$

where M is a constant with an *in vitro value of 210* If a patient rebreaths in a closed system until equilibrium is reached between the patient's blood and the gas in the rebreathing system this equation may be written

$$\frac{S_{CO}}{100 - S_{CO}} = M \cdot \frac{P_{A_{CO}}}{P_{A_{O_2}}} \tag{6}$$

1 —[illegible]

where P_{ACO} and P_{AO_2} are the partial pressure of CO and O_2 respectively in the alveolae and S_{CO} is the CO-saturation in the pulmonary capillaries S_{O_2} may be expressed as $(100—S_{CO})$ since the O_2-saturation of the blood is equal to 100 This equation can be rearranged to express S_{CO} as follows

$$S_{CO} = \frac{M \cdot 100 \cdot P_{ACO}}{P_{AO_2} + M \cdot P_{ACO}} \tag{7}$$

The nitrogen (N_2) is removed from the lungs with pure oxygen to simplify the determination of P_{AO_2} and P_{ACO} According to *Dalton's* law for partial pressures the following equation holds true if P_{AN_2} is approximately equal to 0

$$P_B = P_{AO_2} + P_{ACO} + P_{ACO_2} + P_{AH_2O} \tag{8}$$

The patient is then permitted to rebreath into an oxygen filled system through a carbon dioxide filter until *equilibrium* is reached between patient and rebreathing system with regard to CO This ordinarily takes about 30 minutes When equilibrium is reached it is assumed that

1) $F_{IO_2} \approx 1\ 0$
2) $P_{ACO_2} \approx 40$ mm Hg
3) $P_{AN_2} \approx 0$
4) $RQ = 1\ 0$ i e the number of O_2 molecules leaving the system is the same as the number of CO_2 molecules entering the system
5) an equal number of CO molecules pass through the alveolar membrane in both directions
6) $F_{ACO} = F_{SCO}$

The partial pressure of O_2 in the alveolae P_{AO_2}, can be calculated using Dalton's equation

$$P_{AO_2} \approx F_{AO_2} (P_B - P_{ACO_2} - P_{AH_2O} - P_{ACO}) \tag{9}$$

If P_{ACO} is very low in relation to P_{AO_2}, then P_{ACO} may be made approximately equal to 0

F_{AO_2} is approximately equal to F_{SO_2} in the rebreathing system It is assumed that P_{ACO_2} is approximately equal to 40 mm Hg $P_{AH_2O} = 47$ mm Hg at 37° C and thus equation (9) can be written

$$P_{AO_2} \approx F_{SO_2} (P_B - 87) \tag{10}$$

It is assumed that this holds true for values of $F_{O_2} \geq 0\ 90$

The partial pressure of CO in the alveolae, P_{ACO}

The partial pressure of CO in the alveolae can be calculated in the usual manner as a fraction of the alveolar air at BTPD

$$P_{ACO} = F_{ACO} (P_B - P_{H_2O}) \tag{11}$$

$F_{ACO} \approx F_{SCO}$ and therefore

$$P_{ACO} = F_{SCO} (P_B - 47) \tag{12}$$

If equations (10) and (12) are inserted in equation (7) the following expression is obtained for S_{CO}

$$S_{CO} = \frac{M \cdot 100 \cdot F_{CO} \cdot (P_B - 47)}{F_{O_2} \cdot (P_B - 87) + M \cdot F_{CO} \cdot (P_B - 47)} \quad (13)$$

Determination of amount of CO absorbed, V_{CO}

The amount of CO absorbed by the body after administration can be expressed as

$$V_{CO} = K_{STPD} \cdot [\underbrace{V_{inj} \cdot F_{CO_{inj}}}_{\text{Amount of CO administered}} - \underbrace{(F_{CO_{II}} - F_{CO_I})(V_{APP} + V_{RV})}_{\text{Amount of CO in app. and lungs}} - \underbrace{F_{CO_{II}} \cdot V_S}_{\text{Amount of CO in bag II}}] \quad (14)$$

This expression indicates quite simply that the amount of absorbed CO is equal to the amount of CO administered minus the amount of CO remaining in the apparatus, the rubber bag and the patient's lungs after rebreathing is completed. (See symbols)

Complete equation for calculating THb

If equations (13) and (14) are now inserted in equation (4), the complete equation needed for calculating THb using the alveolar CO method is obtained:

THb g = (15)

$$= \frac{K_{STPD}[V_{inj} \cdot F_{CO_{inj}} - (F_{CO_{II}} - F_{CO_I})(V_{app} + V_{RV}) - F_{CO_{II}} \cdot V_S] \cdot 100 \cdot K_{MV}}{1.34 \left[\frac{M \cdot 100 \cdot F_{CO_{II}}(P_B - 47)}{F_{O_{2II}}(P_B - 87) + M \cdot F_{CO_{II}}(P_B - 47)} - \frac{M \cdot 100 \cdot F_{CO_I}(P_B - 47)}{F_{O_{2I}}(P_B - 87) + M \cdot F_{CO_I}(P_B - 47)} \right]}$$

Approximate equation

The three following measures have been taken to reduce the vast amount of time required to calculate THb by the above method: a list of calculations has been drawn up (see Appendix 2), an ADP program has been written and an approximate equation has been formulated (Linderholm).

There are three prerequisites for using the approximate equation:

P_B = 740—780 mm Hg

$F_{O_2} > 0.90$

Determination is made at room temperature.

The approximate equation is written as follows:

$$THb = \frac{2.60 \cdot V_{inj} \cdot F_{CO_{inj}} \cdot F_{O_{2II}} \cdot K_{PB} \cdot K_T}{1000 \cdot (F_{CO_{II}} - F_{CO_I})} \quad (16)$$

This simplification does not introduce any systematic errors and the coefficient of variation for the difference in values of THb obtained from equations (15) and (16) is 1.1 % if barometric pressure is the only factor for which a correction is intro-

duced Temperature correction is of minor importance relative to pressure correction and it may be disregarded At 21 6° C $K_T = 1\ 00$ A 1° C change in temperature thus changes K_T by 0 003

Calculating the total blood volume

The total blood volume can be calculated from the THb and the hemoglobin concentration as follows

$$TBV = \frac{THb}{10 \times Hb} = \frac{g}{g/100\ ml\ \ 10} = 1 \qquad (17)$$

where Hb is the hemoglobin concentration in g/100 ml of blood

NTIFIKATIONSNR

BESTAMNING AV TDE MED ALVEOLÄRA CO-METODEN

Doc Alf Holmgren Kliniskt Fysiologiska lab

Febr 1966

DECIMALKOMMA STANSAS EJ

41 — FOLKBOKF NR (9–14) – (15–18) — NAMN (19–46)

DIAGNOS (47–78)

42 — REM AVD (9–15) — KÖN (16): Man = 1, Kvinna = 2 — (17): Rokare = 1, Ej rokar = 2 — VIKT KG (18–21) — LANGD CM (22–25)

Tidigare undersökning — ÅR (26–29) — ÅR (30) – MÅN (32) – DAG (35) — FCOINJ 0,995 (36–39) — M 2,3,1 (40–42) — KMY 0,95 (43–45)

43 — Undersokn. I — ÅR (9) – MÅN – DAG (14) — BAROM mm Hg (15–17) — TEMP °C (18–20) — VINJ ml ATPD (21–24) — HB KONC g % (25–28)

Sack 1 — FO2 0 (29–32) — FCO STAND LÅG 00000 (33–40) — STAND CM LÅG (41–44) — PROV CM (45–48)

Säck 2 — FO2 0 (49–52) — FCO STAND HÖG 0000 (53–60) — STAND CM HÖG (61–64) — PROV CM (65–68)

44 — Undersokn. II — ÅR (9) – MÅN – DAG (14) — BAROM mm Hg (15–17) — TEMP °C (18–20) — VINJ ml ATPD (21–24) — HB KONC g (25–28)

Sack 1 — FO2 0 (29–32) — FCO STAND LÅG 00000 (33–40) — STAND CM LÅG (41–44) — PROV CM (45–48)

Sack 2 — FO2 0 (49–52) — FCO STAND HÖG 0000 (53–60) — STAND CM HÖG (61–64) — PROV CM (65–68)

45 — Undersökn III — ÅR (9) – MÅN – DAG (14) — BAROM mm Hg (15–17) — TEMP °C (18–20) — VINJ ml ATPD (21–24) — HB KONC g % (25–28)

Säck 1 — FO2 0 (29–32) — FCO STAND LÅG 00000 (33–40) — STAND CM LÅG (41–44) — PROV CM (45–48)

Sack 2 — FO2 0 (49–52) — FCO STAND HÖG 0000 (53–60) — STAND CM HÖG (61–64) — PROV CM (65–68)

duced Temperature correction is of minor importance relative to pressure correction and it may be disregarded At 21 6° C $K_T = 1\ 00$ A 1° C change in temperature thus changes K_T by 0 003

Calculating the total blood volume

The total blood volume can be calculated from the THb and the hemoglobin concentration as follows

$$TBV = \frac{THb}{10 \times Hb} = \frac{g}{g/100\ ml\ \ 10} = 1 \tag{17}$$

where Hb is the hemoglobin concentration in g/100 ml of blood

BESTAMNING AV THB OCH BLODVOLYM MED CO-METODIK

FØNR 520312- -0 NR 17165 REM AVD

NAMN WIERT ANNA MARGARETA EJ ROKARE

DIAGNOS

TID UNDERS 65 1 14 NR 24

LANGD 153 0 CM VIKT 47 5 KG

DATUM 65 4 5 OCH 6

R E S U L T A T

VARIABEL	NVARDE	OBSVARDE	BEDOMNING	UTLATANDE
TOTAL HAEMOGLOBIN, G	387	394		NORMALT
THB/KR VIKT, G/KG	8 15	8 29		NORMALT
HB, G/100 ML	13 05	14 19	+*	NAGOT HOG
BLODVOLYM, L	3 3	2.8	-*	NAGOT LAG
BV/KR VIKT, ML/KG	69 1	58 4	-*	NAGOT LAG
BLODVOL/0.91, L	3 6	3 0	-*	NAGOT LAG
DITO/KR VIKT, ML/KG	75 9	64 2	-*	NAGOT LAG
INITIAL COHB, PROC	0 47	0 54		NORMALT

ANM

BESTAMNING AV THB MED ALVEOLARA CO-METODEN

FBNR 520312- -0 NR 17165 REM.AVD

NAMN HIERT ANNA MARGARETA EJ ROKARE

DIAGNOS

TID.UNDERS. 65 1 14 NR 2A

LANGD 153.0 CM VIKT 47.5 KG

FCOINJ 0.995 M 231 KMY 0.95

UNDERSOKNING 1 DAT 65 4 5			UNDERSOKNING 2 DAT 65 4 6		
T = 23.5	PB 759 MM HG		T = 23.0	PB 757 MM HG	
VINJ 17.80 ML ATP			VINJ 17.80 ML ATP		
VARIABEL	SACK 1	SACK 2	VARIABEL	SACK 1	SACK 2
FCO * 10^{-7}	648.	1270.	FCO * 10^{-7}	648.	1270.
CM STAND	8 45	15.55	CM STAND	8.65	15.80
CM PROV	2.93	16.10	CM PROV	2.65	16 05
FACO * 10^{-7}	225.	1315.	FACO * 10^{-7}	199.	1290.
PACO * 10^{-4}	160.	936.	PACO * 10^{-4}	141.	916.
FAO2	0.957	0.940	FAO2	0 954	0 940
PAO2 MM HG	643.	632	PAO2 MM HG	639.	630.
COHB PROC	0.57	3.31	COHB PROC	0.51	3.25

DCOHB 2.74 THB 394. DCOHB 2.74 THB 393.

HB 14.29 BV 2.76 HB 14.10 BV 2.79

LINEARITETSKVOT 1.07 1.07

BESTAMNING AV THB OCH BLODVOLYM MED CO-METODIK

FBNR 520312- -0 NR 17165 REP AVD

NAMN WIERT ANNA MARGARETA EJ ROKARE

DIAGNOS

TID UNDERS 65 1 14 NR 2A

LANGD 153 0 CM VIKT 47 5 KG

DATUM 65 4 5 OCH 6

R E S U L T A T

VARIABEL	NVARDE	OBSVARDE	BEDOMNING	UTLATANDE
TOTAL HAEMOGLOBIN, G	387	394		NORMALT
THB/KR VIKT, G/KG	8 15	8 29		NORMALT
HB, G/100 ML	13 05	14 19	+*	NAGOT HOG
BLODVOLYM, L	3 3	2 8	-*	NAGOT LAG
BV/KR VIKT, ML/KG	69 1	58 4	-*	NAGOT LAG
BLODVOL/0 91, L	3 6	3 0	-*	NAGOT LAG
DITO/KR VIKT, ML/KG	75 9	64 2	-*	NAGOT LAG
INITIAL COHB, PROC.	0 47	0 54		NORMALT

ANM

Calculation

Calculation of the total amount of hemoglobin in the body using *Sjostrand's alveolar carbon monoxide method*

1 Patient identification, certain standard information such as, height, diagnosis, smoking habits, previous investigations etc

2 Input data common to all measurements

(1) FCOINJ = x xxx

(2) M = xxx

(3) KMY = x xx

3 FIRST MEASUREMENT I

Input data common to both rebreathing bags

(4) TEMP = xx x °C

(5) PB = xxx mm Hg

(6) VINJ = xx xx ml ATP

(7) HBKONC = xx xx g-procent

Input data, bag 1

(8) FO2 = x xxx

(9) FCOSTAND low = x xxxxxxx

(10) CM STAND low = xx xx

(11) CM BAG 1 = xx xx

Computed data, bag 1

(12) PAO2 = (8) x [(5) - 87] = FO2 (PB - 87) = xxx, mm Hg

(13) FCO $= \dfrac{(9) \times (11)}{(10)} = x\ xxxxxxx = \dfrac{\text{FCO STAND} \times \text{CM BAG}}{\text{CM STAND}}$

(14) PACO $= (13) \times [(5) - 47] = \text{FCO} \times [\text{PB} - 47]$, mm Hg

(15) COHB 1 $= \dfrac{(2) \times (14) \times 100}{(12) + (2) \times (14)} = \dfrac{\text{M} \times \text{PACO} \times 100}{\text{PAO2} + \text{M} \times \text{PACO}} = x\ xx$, %

<u>Input data, bag 2</u>

(16) FO2 = x xxx

(17) FCO STAND high = x xxxxxxx

(18) CM STAND high = xx xx

(19) CM BAG 2 = xx xx

<u>Computed data, bag 2</u>

(20) PAO2 $= (16) \times [(5) - 87] = \text{FO2}\ [\text{PB} - 87] = xxx$, mm Hg

(21) FCO $= \dfrac{(17) \times (19)}{(18)} = x\ xxxxxxx = \dfrac{\text{FCO STAND} \times \text{CM BAG}}{\text{CM STAND}}$

(22) PACO $= (21) \times [(5) - 47] = \text{FCO}\ [\text{PB} - 47]$, mm Hg

(23) COHB 2 $= \dfrac{(2) \times (22) \times 100}{(20) + (2) \times (22)} = \dfrac{\text{M} \times \text{PACO} \times 100}{\text{PAO2} + \text{M} \times \text{PACO}} = x\ xx$, %

<u>Calculation of THb</u>

(24) Δ COHB $= (23) - (15) = x\ xx$ COHB 2 − COHB 1, %

(25) $= (21) \times 9500 = \text{FCO 2} \times 9500$

(26) $= (13) \times 4500 = \text{FCO 1} \times 4500$

(27) $= (25) - (26)$

(28) CO UPTAKE $= (1) \times (6) - (27) = \text{FCOINJ} \times \text{VINJ} - \text{CO-loss}$

(29) THB I $= \dfrac{(3) \times 100}{1\ 34} \times \dfrac{273 \times (5)}{(273 + (4))\ 760} \times \dfrac{(28)}{(24)} = xxxx$, g

Calculation

Calculation of the total amount of hemoglobin in the body using Sjostrand's alveolar carbon monoxide method

1 Patient identification, certain standard information such as, height, diagnosis, smoking habits, previous investigations etc

2 Input data common to all measurements

(1) FCOINJ = x xxx

(2) M = xxx

(3) KMY = x xx

3 FIRST MEASUREMENT I

Input data common to both rebreathing bags

(4) TEMP = xx x °C

(5) PB = xxx mm Hg

(6) VINJ = xx xx ml ATP

(7) HBKONC = xx xx g-procent

Input data, bag I

(8) FO2 = x xxx

(9) FCOSTAND low = x xxxxxxx

(10) CM STAND low = xx xx

(11) CM BAG 1 = xx xx

Computed data, bag 1

(12) PAO2 = (8) x [(5) - 87] = FO2 (PB - 87) = xxx, mm Hg

```
EMOGLOBIN DOC  HOLMGREN                     FORTRAN SOURCE LIST
 ISN        SOURCE STATEMENT

   0 $IBFTC ALFA     NOLIST,NOREF
     C
     C       BERAKNING AV HAMOGLOBINMANGD MED -ALVEOLARA CO-METODEN-
     C
   1         DIMENSION DIAG(6),ANAMN(5),IFBNR(2),IDAT(3),IDAT1(3),IDAT2(3),
            1IDAT3(3),ALPHA(7),TEXT(29),X1(12),X2(12),X3(12),V1(16),V2(16),
            2V3(16) V4(59),AVO(2)
   2         DATA ALPHA/42H+*** +**   +*        -*     -**    -***   /
   3         DATA TEXT/174HMYCKET HOG  HOG    NAGOT HOG    NAGOT LAG    LAG    MYCK
            1ET LAG            ALINEARITET LACKAGE      ROKARE    HAEMOLYS TRE
            2 BESTAMNINGSARNORMALT      ELLER OKAND CO-EXPOSITION       /
    C
    C                 INLASNING AV PATIENTKORT 41
    C
   4         NRSEN=0
   5       2 READ(5,3)IDNR,KTYP
  10       3 FORMAT(I6,I2)
  11       4 IF(KTYP-99)5,38,5
  12       5 IF(IDNR-NRSEN)41,2,6
  13       6 NRSEN=IDNR
  14         IF(KTYP-41)44,8,44
  15       8 CALL REREAD
  16         READ(5,10)IDNR1,KTYP,IFBNR,(ANAMN(I),I=1,5),(DIAG(I),I=1,6)
  32      10 FORMAT(I6,I2,I6,I4,4A6,A4,5A6,A2)
     C
     C                 INLASNING AV PATIENTKORT 42
     C
  33         READ(5,3)IDNR,KTYP
  36         IF(KTYP-99)13,38,13
  37      13 IF(IDNR-NRSEN)41,14,47
  40      14 IF(KTYP-42)50,15,50
  41      15 CALL REREAD
  42         READ(5,17)IDNR1,KTYP,(AVO(I),I=1,2),IK,IR,VIKT,YLNGD,
            1ANUM,IDAT,FLOINJ,YH,YKMY
  54      17 FORMAT(I6,I2,A6,A1 2I1,2F4 1 A4,3I2,F4 3,F3 0,F3 2)
     C
     C                 INLASNING AV UNDERSOKNINGSKORT 1  KTYP 43
     C
  55         READ(5,3)IDNR,KTYP
  60         IF(KTYP-99)20,38,20
  61      20 IF(IDNR-NRSEN)41,21,47
  62      21 IF(KTYP-43)52,22,52
  63      22 CALL REREAD
  64         READ(5,24)IDNR1 KTYP,IDAT1,(X1(I),I=1,12)
  74      24 FORMAT(I6,I2,3I2,F3 0,F3 1,2F4 2,2(F4 3,F8 7,2F4 2))
  75         GOTO100
      C
      C                INLASNING AV UNDERSOKNINGSKORT 2  KTYP 44
      C
  76      26 READ(5,3)IDNR KTYP
 101         IF(IDNR-NRSEN)41,28,400
 102      28 IF(KTYP-44)400,29,400
 103      29 CALL REREAD
 104         READ(5,24)IDNR1 KTYP,IDAT2,(X2(I),I=1,12)
 114         GOTO200
```

BV = (29) / (7) 10 = xx xx, 1

4 SECOND MEASUREMENT II

Input data common to both rebreathing bags

TEMP = xx.x °C

PB = xxx mm Hg

VINJ = xx xx ml ATP

HBKONC = xx xx g-procent

The remainder of the THB II calculation is the same as for THB I

Calculations for printout

THB, g = (THb I + THB II) / 2 = xxx

THB/KG = g/KG = xx xx

PREDICTED THB, g = xxxx

BV, 1 = (BV I + BV II)/2 = xx x

BV/KG ml/KG = xx x

PREDICTED BV, 1 = xx.x

HB, g % = (HB I + HB II)/2 = xx x

HB NORMAL VALUE, g % = xx xx

INITIAL COHB = % (COHB 1 I + COHB 1 II)/2 = x xx

INITIAL COHB NORMAL VALUE % = x xx

Calculations for methodological controls

I FO2 if FO2 < 0 90 in any bag, print LEAKAGE = LACKAGE

II COHB if COHB > 1 0 % and the patient is a smoker, print SMOKER = RÖKARE

and if the patient does not smoke, print HEMOLYSES

OR UNKNOWN EXPOSURE TO CO = HAMOLYS ELLER OKAND CO-EXPOSITION

```
HAEMOGLOBIN DOC  HOLMGREN                    FORTRAN SOURCE LIST
   ISN        SOURCE STATEMENT

     0 $IBFTC ALFA    NOLIST,NOREF
       C
       C       BERAKNING AV HAMOGLOBINMANGD MED -ALVEOLARA CO-METODEN-
       C
     1          DIMENSION DIAG(6),ANAMN(5),IFBNR(2),IDAT(3),IDAT1(3),IDAT2(3),
              1IDAT3(3),ALPHA(7),TEXT(29),X1(12),X2(12),X3(12),V1(16),V2(16),
              2V3(16),V4(59),AVD(2)
     2          DATA ALPHA/42H+***  +**    +*          -*     -**    -***  /
     3          DATA TEXT/174HMYCKET HOG  HOG    NAGOT HOG    NAGOT LAG    LAG   MYCK
              1ET LAG             ALINEARITET LACKAGE      ROKARE    HAEMOLYS TRE
              2  BESTAMNINGARNORMALT      ELLER OKAND CO-EXPOSITION      /
      C
      C                 INLASNING AV PATIENTKORT 41
      C
     4          NRSEN=0
     5        2 READ(5,3)IDNR,KTYP
    10        3 FORMAT(I6,I2)
    11        4 IF(KTYP-99)5,38,5
    12        5 IF(IDNR-NRSEN)41,2,6
    13        6 NRSEN=IDNR
    14          IF(KTYP-41)44,8,44
    15        8 CALL REREAD
    16          READ(5,10)IDNR1,KTYP,IFBNR,(ANAMN(I),I=1,5),(DIAG(I),I=1,6)
    32       10 FORMAT(I6,I2,I6,I4,4A6,A4,5A6,A2)
       C
       C                 INLASNING AV PATIENTKORT 42
       C
    33          READ(5,3)IDNR,KTYP
    36          IF(KTYP-99)13,38,13
    37       13 IF(IDNR-NRSEN)41,14,47
    40       14 IF(KTYP-42)50,15,50
    41       15 CALL REREAD
    42          READ(5,17)IDNR1,KTYP,(AVD(I),I=1,2),IK,IR,VIKT,YLNGD,
               1ANUM,IDAT,FCOINJ,YM,YKMY
    54       17 FORMAT(I6,I2,A6,A1,2I1,2F4 1,A4,3I2,F4 3,F3 0,F3 2)
        C
        C                INLASNING AV UNDERSOKNINGSKORT 1  KTYP 43
        C
    55          READ(5,3)IDNR,KTYP
    60          IF(KTYP-99)20,38,20
    61       20 IF(IDNR-NRSEN)41,21,47
    62       21 IF(KTYP-43)52,22,52
    63       22 CALL REREAD
    64          READ(5,24)IDNR1,KTYP,IDAT1,(X1(I),I=1,12)
    74       24 FORMAT(I6,I2,3I2,F3 0,F3 1,2F4 2,2(F4 3,F8 7,2F4 2))
    75          GOTO100
         C
         C               INLASNING AV UNDERSOKNINGSKORT 2  KTYP 44
         C
    76       26 READ(5 3)IDNR,KTYP
   101          IF(IDNR-NRSEN)41,28,400
   102       28 IF(KTYP-44)400,29,400
   103       29 CALL REREAD
   104          READ(5,24)IDNR1,KTYP,IDAT2,(X2(I),I=1,12)
   114          GOTO200
```

BV = $\frac{(29)}{(7)\ 10}$ = xx xx, 1

4 SECOND MEASUREMENT II

Input data common to both rebreathing bags

TEMP = xx x ^oC

PB = xxx mm Hg

VINJ = xx xx ml ATP

HBKONC = xx xx g-procent

The remainder of the THB II calculation is the same as for THB I.

Calculations for printout

THB, g = $\frac{\text{THb I + THB II}}{2}$ = xxx

THB/KG = g/KG = xx xx

PREDICTED THB, g = xxxx

BV, I = (BV I + BV II)/2 = xx x

BV/KG ml/KG = xx x

PREDICTED BV, 1 = xx x

HB, g 7 = (HB I + HB II)/2 = xx x

HB NORMAL VALUE, g 7 = xx xx

INITIAL COHB = 7 (COHB 1 I + COHB 1 II)/2 = x,xx

INITIAL COHB NORMAL VALUE 7 = x xx

Calculations for methodological controls

I FO2 if FO2 < 0 90 in any bag, print LEAKAGE = LACKAGE

II COHB if COHB > 1 0 7 and the patient is a smoker, print SMOKER = RÖKARE

and if the patient does not smoke, print HEMOLYSES

OR UNKNOWN EXPOSURE TO CO = HÄMOLYS ELLER OKAND CO-EXPOSITION

```
HAEMOGLOBIN DOC  HOLMGREN                    FORTRAN SOURCE LIST
   ISN         SOURCE STATEMENT

    0 $IBFTC ALFA     NOLIST,NOREF
      C
      C        BERAKNING AV HAMOGLOBINMANGD MED -ALVEOLARA CO-METODEN-
      C
    1          DIMENSION DIAG(6),ANAMN(5),IFBNR(2),IDAT(3),IDAT1(3),IDAT2(3),
              1IDAT3(3),ALPHA(7),TEXT(29),X1(12),X2(12),X3(12),V1(16),V2(16),
              2V3(16),V4(59),AVD(2)
    2          DATA ALPHA/42H+***  +**   +*         -*    -**   -***  /
    3          DATA TEXT/174HMYCKET HOG  HOG   NAGOT HOG   NAGOT LAG   LAG   MYCK
              1ET LAG            ALINEARITET LACKAGE      ROKARE    HAEMOLYS TRE
              2 BESTAMNINGARNORMALT     ELLER OKAND CO-EXPOSITION        /
     C
     C                  INLASNING AV PATIENTKORT 41
     C
    4          NRSEN=0
    5        2 READ(5,3)IDNR,KTYP
   10        3 FORMAT(I6,I2)
   11        4 IF(KTYP-99)5,38,5
   12        5 IF(IDNR-NRSEN)41,2,6
   13        6 NRSEN=IDNR
   14          IF(KTYP-41)44,8,44
   15        8 CALL REREAD
   16          READ(5,10)IDNR1,KTYP,IFBNR,(ANAMN(I),I=1,5),(DIAG(I),I=1,6)
   32       10 FORMAT(I6,I2,I6,I4,4A6,A4,5A6,A2)
      C
      C                  INLASNING AV PATIENTKORT 42
      C
   33          READ(5,3)IDNR KTYP
   36          IF(KTYP-99)13,38,13
   37       13 IF(IDNR-NRSEN)41,14,47
   40       14 IF(KTYP-42)50,15,50
   41       15 CALL REREAD
   42          READ(5,17)IDNR1,KTYP,(AVD(I),I=1,2),IK,IR,VIKT,YLNGD,
              1ANUM,IDAT,FCOINJ,YM YKMY
   54       17 FORMAT(I6,I2,A6,A1,2I1,2F4 1,A4,3I2,F4 3,F3 0,F3 2)
       C
       C                  INLASNING AV UNDERSOKNINGSKORT 1  KTYP 43
       C
   55          READ(5,3)IDNR,KTYP
   60          IF(KTYP-99)20 38,20
   61       20 IF(IDNR-NRSEN)41,21,47
   62       21 IF(KTYP-43)52,22 52
   63       22 CALL REREAD
   64          READ(5,24)IDNR1 KTYP,IDAT1,(X1(I),I=1,12)
   74       24 FORMAT(I6,I2,3I2,F3 0,F3 1,2F4 2,2(F4 3,F8 7,2F4 2))
   75          GOTO100
       C
       C                  INLASNING AV UNDERSOKNINGSKORT 2  KTYP 44
       C
   76       26 READ(5 3)IDNR,KTYP
  101          IF(IDNR-NRSEN)41,28,400
  102       28 IF(KTYP-44)400,29,400
  103       29 CALL REREAD
  104          READ(5 24)IDNR1,KTYP,IDAT2,(X2(I),I=1,12)
  114          GOTO200
```

$$BV = \frac{(29)}{(7)\ 10} = xx\ xx,\ l$$

4 SECOND MEASUREMENT II

Input data common to both rebreathing bags

TEMP = xx x °C

PB = xxx mm Hg

VINJ = xx xx ml ATP

HBKONC = xx xx g-procent

The remainder of the THB II calculation is the same as for THB I

Calculations for printout

$$THB,\ g = \frac{\text{THb I + THB II}}{2} = xxx$$

THB/KG = g/KG = xx xx

PREDICTED THB, g = xxxx

BV, l = (BV I + BV II)/2 = xx x

BV/KG ml/KG = xx x

PREDICTED BV, l = xx x

HB, g % = (HB I + HB II)/2 = xx x

HB NORMAL VALUE, g % = xx xx

INITIAL COHB = % (COHB 1 I + COHB 1 II)/2 = x xx

INITIAL COHB NORMAL VALUE % = x xx

Calculations for methodological controls

I FO2 if FO2 < 0 90 in any bag, print LEAKAGE = LACKAGE

II COHB if COHB > 1 0 % and the patient is a smoker, print SMOKER = ROKARE

and if the patient does not smoke, print HEMOLYSES OR UNKNOWN EXPOSURE TO CO = HAMOLYS ELLER OKAND CO-EXPOSITION

```
HAEMOGLOBIN DOC  HOLMGREN                     FORTRAN SOURCE LIST
   ISN      SOURCE STATEMENT

    0 $IBFTC ALFA     NOLIST,NOREF
      C
      C       BERAKNING AV HAMOGLOBINMANGD MED -ALVEOLARA CO-METODEN-
      C
    1          DIMENSION DIAG(6),ANAMN(5),IFBNR(2),IDAT(3),IDAT1(3),IDAT2(3),
             1IDAT3(3),ALPHA(7),TEXT(29),X1(12),X2(12),X3(12),V1(16),V2(16),
             2V3(16),V4(59),AVD(2)
    2          DATA ALPHA/42H++++  +++   ++          -+    -++   -+++  /
    3          DATA TEXT/174HMYCKET HOG  HOG   NAGOT HOG   NAGOT LAG   LAG   MYCK
             1ET LAG            ALINEARITET LACKAGE       ROKARE    HAEMOLYS TRE
             2  BESTAMNINGARNORMALT      ELLER OKAND CO-EXPOSITION      /
     C
     C                  INLASNING AV PATIENTKORT 41
     C
    4          NRSEN=0
    5        2 READ(5,3)IDNR,KTYP
   10        3 FORMAT(I6,I2)
   11        4 IF(KTYP-99)5,38,5
   12        5 IF(IDNR-NRSEN)41,2,6
   13        6 NRSEN=IDNR
   14          IF(KTYP-41)44,8,44
   15        8 CALL REREAD
   16          READ(5,10)IDNR1,KTYP,IFBNR,(ANAMN(I),I=1,5),(DIAG(I),I=1,6)
   32       10 FORMAT(I6,I2,I6,I4,4A6,A4,5A6,A2)
      C
      C                 INLASNING AV PATIENTKORT 42
      C
   33          READ(5,3)IDNR,KTYP
   36          IF(KTYP-99)13,38,13
   37       13 IF(IDNR-NRSEN)41,14,47
   40       14 IF(KTYP-42)50,15,50
   41       15 CALL REREAD
   42          READ(5,17)IDNR1,KTYP,(AVD(I),I=1,2),IK,IR,VIKT,YLNGD,
              1ANUM,IDAT,FCOINJ,YM,YKMY
   54       17 FORMAT(I6,I2,A6,A1,2I1,2F4 1,A4,3I2,F4 3,F3 0,F3 2)
       C
       C                 INLASNING AV UNDERSOKNINGSKORT 1  KTYP 43
       C
   55          READ(5,3)IDNR,KTYP
   60          IF(KTYP-99)20,38,20
   61       20 IF(IDNR-NRSEN)41,21,47
   62       21 IF(KTYP-43)52,22 52
   63       22 CALL REREAD
   64          READ(5,24)IDNR1 KTYP,IDAT1,(X1(I),I=1,12)
   74       24 FORMAT(I6,I2,3I2,F3 0,F3 1,2F4 2,2(F4 3,F8 7,2F4 2))
   75          GOTO100
        C
        C                INLASNING AV UNDERSOKNINGSKORT 2  KTYP 44
        C
   76       26 READ(5 3)IDNR,KTYP
  101          IF(IDNR-NRSEN)41,28,400
  102       28 IF(KTYP-44)400,29,400
  103       29 CALL REREAD
  104          READ(5 24)IDNR1 KTYP,IDAT2,(X2(I),I=1,12)
  114          GOTO200
```

BV = $\frac{(29)}{(7)\ 10}$ = xx xx, 1

4 SECOND MEASUREMENT II

Input data common to both rebreathing bags

TEMP = xx x °C

PB = xxx mm Hg

VINJ = xx xx ml ATP

HBKONC = xx xx g-procent

The remainder of the THB II calculation is the same as for THB I

Calculations for printout

THB, g = $\frac{\text{THb I + THB II}}{2}$ = xxx

THB/KG = g/KG = xx xx

PREDICTED THB, g = xxxx

BV, 1 = (BV I + BV II)/2 = xx x

BV/KG ml/KG = xx x

PREDICTED BV, 1 = xx x

HB, g 7 = (HB I + HB II)/2 = xx x

HB NORMAL VALUE, g 7 = xx xx

INITIAL COHB = 7 (COHB 1 I + COHB 1 II)/2 = x xx

INITIAL COHB NORMAL VALUE 7 = x xx

Calculations for methodological controls

I FO2 if FO2 < 0 90 in any bag, print LEAKAGE = LACKAGE

II COHB if COHB > 1 0 7 and the patient is a smoker, print SMOKER = RÖKARE

and if the patient does not smoke, print HEMOLYSES OR UNKNOWN EXPOSURE TO CO = HAMOLYS ELLER OKAND CO-EXPOSITION

```
HOGLOBIN DOC  HOLMGREN                  FORTRAN SOURCE LIST
ISN       SOURCE STATEMENT

  0 $IBFTC ALFA     NOLIST,NOREF
    C
    C       BERAKNING AV HAMOGLOBINMANGD MED -ALVEOLARA CO-METODEN-
    C
  1         DIMENSION DIAG(6),ANAMN(5),IFBNR(2),IDAT(3),IDAT1(3),IDAT2(3),
           1IDAT3(3),ALPHA(7),TEXT(29),X1(12),X2(12),X3(12),V1(16),V2(16),
           2V3(16),V4(59),AVD(2)
  2         DATA ALPHA/42H++++  +++    ++            -+     -++    -+++   /
  3         DATA TEXT/174HMYCKET HOG  HOG    NAGOT HOG    NAGOT LAG    LAG    MYCK
           1ET LAG              ALINEARITET LACKAGE      ROKARE      HAEMOLYS TRE
           2  BESTAMNINGARNORMALT       ELLER OKAND CO-EXPOSITION        /
    C
    C                 INLASNING AV PATIENTKORT 41
    C
  4         NRSEN=0
  5       2 READ(5,3)IDNR,KTYP
 10       3 FORMAT(I6,I2)
 11       4 IF(KTYP-99)5,38,5
 12       5 IF(IDNR-NRSEN)41,2,6
 13       6 NRSEN=IDNR
 14         IF(KTYP-41)44,8,44
 15       8 CALL REREAD
 16         READ(5,10)IDNR1,KTYP,IFBNR,(ANAMN(I),I=1,5),(DIAG(I),I=1,6)
 32      10 FORMAT(I6,I2,I6,I4,4A6,A4,5A6,A2)
    C
    C                 INLASNING AV PATIENTKORT 42
    C
 33         READ(5,3)IDNR,KTYP
 36         IF(KTYP-99)13,38,13
 37      13 IF(IDNR-NRSEN)41,14,47
 40      14 IF(KTYP-42)50,15,50
 41      15 CALL REREAD
 42         READ(5,17)IDNR1,KTYP,(AVD(I),I=1,2),IK,IR,VIKT,YLNGD,
           1ANUM,IDAT,FCOINJ,YM,YKMY
 54      17 FORMAT(I6,I2,A6,A1,2I1,2F4 1,A4,3I2,F4.3,F3 0,F3 2)
    C
    C                 INLASNING AV UNDERSOKNINGSKORT 1  KTYP 43
    C
 55         READ(5,3)IDNR,KTYP
 60         IF(KTYP-99)20,38,20
 61      20 IF(IDNR-NRSEN)41,21,47
 62      21 IF(KTYP-43)52,22,52
 63      22 CALL REREAD
 64         READ(5,24)IDNR1,KTYP IDAT1,(X1(I),I=1,12)
 74      24 FORMAT(I6,I2,3I2,F3 0,F3 1,2F4 2,2(F4 3,F8 7,2F4 2))
 75         GOTO100
    C
    C                 INLASNING AV UNDERSOKNINGSKORT 2  KTYP 44
    C
 76      26 READ(5 3)IDNR,KTYP
101         IF(IDNR-NRSEN)41 28,400
102      28 IF(KTYP-44)400,29,400
103      29 CALL REREAD
104         READ(5,24)IDNR1,KTYP,IDAT2,(X2(I),I=1,12)
114         GOTO200
```

```
HAEMOGLOBIN DOC. HOLMGREN                    FORTRAN SOURCE LIST ALFA
  ISN        SOURCE STATEMENT

      C
      C                INLASNING AV UNDERSOKNINGSKORT 3  KTYP 45
      C
  115      32 READ(5,3)IDNR,KTYP
  120         IF(IDNR-NRSEN)41,34,500
  121      34 IF(KTYP-45)500,35,500
  122      35 CALL REREAD
  123         READ(5,24)IDNR1,KTYP,IDAT3,(X3(I),I=1,12)
  133         GOTO300
      C
      C                UTSKRIFT AV SLUT- OCH FELMEDDELANDE
      C
  134      38 WRITE(6,39)
  135      39 FORMAT(21H1 BEARBETNINGEN  SLUT)
  136         STOP
  137      41 WRITE(6,42)IDNR
  140      42 FORMAT(44H1 EDRA DATAKORT AR FELSORTERADE. IDENTNR  = ,I6)
  141         STOP
  142      44 WRITE(6,45)IDNR
  143      45 FORMAT(34H1 KORTTYP 41 SAKNAS FOR PATIENT = ,I6)
  144         GOTO2
  145      47 WRITE(6,48)IDNR
  146      48 FORMAT(41H1 UNDERSOKNINGSKORT SAKNAS FOR PATIENT = ,I6)
  147         GOTO2
  150      50 WRITE(6,51)IDNR
  151      51 FORMAT(34H1 KORTTYP 42 SAKNAS FOR PATIENT = ,I6)
  152         GOTO2
  153      52 WRITE(6,53)IDNR
  154      53 FORMAT(34H1 KORTTYP 43 SAKNAS FOR PATIENT = ,I6)
  155         GOTO2
      C
      C                BERAKNINGAR FOR UNDERSOKNING 1
      C
  156     100 V1(1)=X1(5)*(X1(1)-87.)
  157         IF(X1(7) EQ.0.)GOTO140
  162         V1(2)= X1(6)*X1(8)/X1(7)
  163         V1(3)=V1(2)*(X1(1)-47.)
  164         V1(4)=(YM*V1(3)*100 )/(V1(1)+YM*V1(3))
  165     107 V1(5)=X1(9)*(X1(1)-87.)
  166         IF(X1(11) EQ 0.)GOTO144
  171         V1(6)=(X1(10)*X1(12))/X1(11)
  172         V1(7)=V1(6)*(X1(1)-47.)
  173         V1(8)=(YM*V1(7)*100 )/(V1(5)+YM*V1(7))
  174     114 V1(9)=V1(8)-V1(4)
  175         V1(10)=V1(6)*9500.
  176         V1(11)=V1(2)*4500.
  177         V1(2)=V1(2)*10000000
  200         V1(3)=V1(3)*10000
  201         V1(6)=V1(6)*10000000
  202         V1(7)=V1(7)*10000
  203         V1(12)=V1(10)-V1(11)
  204         V1(13)=FCOINJ*X1(3)-V1(12)
  205         V1(14)=(YKMY*100 *273 *X1(1)*V1(13))/(1.34*(273 *X1(2))*760 *V1(9)
             1)
  206         IF(X1(4).EQ 0 )GOTO148
```

From the Departments of Physiology Graduate Division and Medicine School of Medicine
University of Pennsylvania Philadelphia Pennsylvania

Endogenous Carbon Monoxide Production and Body CO Stores

By

RONALD F COBURN[1]

T Sjostrand is considered to be the founder of the rapidly expanding field related to endogenous CO production This investigator was the first to present direct evidence in support of the concept that blood CO in part originates from body metabolic processes (22) In addition he observed that blood CO levels are elevated in patients with hemolytic anemia (21) This led to the hypothesis that CO might orginate as a catabolic by product of hemoglobin arising from a heme bridge carbon atom and Sjostrand was able to demonstrate that chemical oxidation of hemoglobin results in CO formation (23) Subsequent studies performed in our laboratory have confirmed the existence of endogenously produced CO in normal man (1 3) Likewise the concept that CO originates during hemoglobin catabolism from a *heme bridge carbon atom* has been supported by recent experiments (3 8)

Recently there have been further advances in our understanding of the variables that influence endogenous CO production and the relationships of CO production and the body CO stores A method of measuring the rate of CO production has been developed (1) which allows a quantitative approach to the relationships of hemoglobin catabolism and CO production Relationships of blood carboxyhemoglobin percent saturation rate of CO production, rate of excretion, and rate of CO metabolism have been clarified (4 6) In the present paper we have reviewed some of these studies We have included new data from experiments designed to explore the possibility that skin CO exchange or exchanges between the body s formate and CO pools might significantly influence the body CO stores

Methods

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Mongrel dogs were anesthetized with pentobarbital and isolated from environmental air by placing them in a

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        C                   INLASNING AV UNDERSOKNINGSKORT 3  KTYP 45
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   133         GOTO300
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        C                   UTSKRIFT AV SLUT- OCH FELMEDDELANDE
        C
   134      38 WRITE(6,39)
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 ISN         SOURCE STATEMENT

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      C                  INLASNING AV UNDERSOKNINGSKORT 3  KTYP 45
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 121       34 IF(KTYP-45)500,35,500
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 141          STOP
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 143       45 FORMAT(34H1 KORTTYP 41 SAKNAS FOR PATIENT = ,I6)
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```

Fig 2 Changes in the body CO stores in an anesthetized dog before and after 0 5 % CO was added to the gas in the box in contact with skin and mucous membranes There was no change in the rate of increase in body CO indicating that skin CO uptake was negligible in this experiment

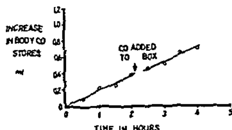
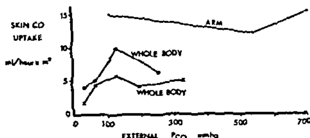


Fig 3 Skin CO uptake in man The rate of uptake of CO is expressed in ml per min × m² versus the transdermal Pco gradient



trations of box gas and gas in the rebreathing system were measured with a gas chromatograph Two experiments were performed on 2 dogs

In another series of experiments skin CO transport was studied in normal men In two experiments the subject was placed in a large rubber bag The bag was sealed with colostomy cement leaving one arm and the head exposed The bag was fitted with tubes for rapid addition and removal of gas mixtures The subject breathed through a mouth piece connected to a rebreathing system similar to that described above except that no respirator was included The subject wore only underpants In one experiment a bag was sealed about the left arm of a male subject Baseline blood samples were drawn for [COHb] and the bags inflated with varying concentrations of CO (5 to 100 %) in N_2 After 15 minutes the gas was removed the bag flushed with air and a second venous blood sample drawn for [COHb] The quantity of CO absorbed was calculated from the increase in [COHb] and CO dilution which in turn was determined by adding a known quantity of CO to the rebreathing system and measuring the resultant increase in [COHb] Several runs at varying bag CO % were made in each experiment Since skin CO transport was relatively great in these experiments no correction was made for endogenous CO production

Radioactive Sodium Formate Injection Experiments

Two dogs were anesthetized with pentobarbital and attached to a rebreathing system as described above Sodium formate C^{14} 100 μc was injected intravenously Blood samples were drawn periodically for 4 hours and analyzed for $C^{14}O$ (6 17) by extracting CO from

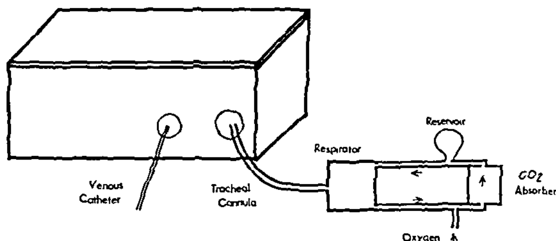


Fig 1 Preparation used in the dog skin CO uptake experiments The apparatus consisted of a box and rebreathing system and was designed so that a constant CO concentration could be maintained in box gas without contamination of the gas in the rebreathing system

box which was then sealed The animals breathed via a balloon tracheal cannula which led through the wall of the box to a rebreathing system A catheter for blood sampling was placed in the external jugular vein and also led to the outside of the box The rate of endogenous CO production (Vco) (1) was determined from the rate of increase in carboxyhemoglobin in venous blood during the first two hours of the experiment CO was then added to gas in the box giving a concentration of approximately 0 5 % Ne was also added so that the possibility of leaks from gas in the box into the rebreathing system could be evaluated Venous blood samples were then collected every half hour for a second two hour period Since endogenous CO production is constant within the error of the measurement (1, 6) if CO was entering the body gas stores via skin or mucous membranes the rate of increase of body stores would be greater than explainable by endogenous CO production The rate of skin uptake, therefore, could be calculated by subtracting the control rate of increase in body CO stores from the measured rate determined after CO was added to the box

The apparatus used in the experiments is illustrated in Figure 1 The box was constructed of one half inch plastic and measured 12 × 12 × 30 inches The top of the box was sealed with rubber and clamped tightly The tracheal cannula and venous catheter were led out through rubber stoppers placed in 2 inch diameter holes in the wall of the box The rebreathing system consisted of a respirator, CO_2 absorber, O_2 demand valve and rubber bag that acted as a gas reservoir The PO_2 in the rebreathing system was maintained at 150—160 mmHg Alveolar ventilation was regulated to maintain a normal arterial Pco_2 Blood carboxyhemoglobin [COHb] was measured with an infrared method (2) that can detect ± (SD) 0 02 % saturation The Neon and CO concen

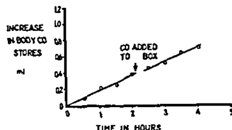


Fig 2 Changes in the body CO stores in an anesthetized dog before and after 0.5 % CO was added to the gas in the box in contact with skin and mucous membranes There was no change in the rate of increase in body CO indicating that skin CO uptake was negligible in this experiment

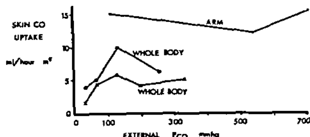


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a 2 ml blood sample and transferring it into an evacuated ionization chamber The method can detect 1×10^{-4} μc

Results

Skin CO Exchange Experiments

Results from an experiment where approximately 0 5 % CO was placed in contact with skin and mucous membranes of an anesthetized dog are shown in Figure 2 In two experiments the rate of increase of body CO stores did not change compared to the control, therefore, significant quantities of CO did not traverse the skin or mucous membranes The ratio of CO/Ne in box air did not change during the experiment and significant quantities of Ne were not found in the gas in the rebreathing system

The body CO stores increased markedly in all three experiments on human subjects indicating uptake of CO via the skin Data are shown in Figure 3 Skin uptake was apparently independent of external Pco over the range 100—700 mmHg At external Pco less than 100 mmHg skin uptake appeared to be proportional to external Pco In the experiment where CO was in contact with the skin of the right arm, CO uptake was significantly greater at equivalent external Pco when expressed in terms of ml/hr $\times$ m^2 than in the two experiments where 90 % of the body's skin was exposed to CO

Formate Injection Experiments

No radioactivity was measureable in the body CO stores up to 4 hours following injection of sodium formate C^{14}

Discussion

Processes That Influence Body CO Stores and Blood Carboxyhemoglobin % Saturation

It seems obvious that the rate of endogenous CO production is a primary determinant of the body CO stores and blood carboxyhemoglobin % saturation, however, the other variables involved that influence the body CO stores and the interaction of these variables have rarely been considered The physiological variables that are thought to influence body CO (4, 6, 17) include Vco, rate of metabolism of body CO, the variables that determine excretion of CO in the lung, variables that influence uptake of CO from the environment, and variables that influence distribution of CO in the body In the present study we have investigated the possibility that skin CO transport or exchanges of body CO with body formate might also be significant variables

Studies of changes in the body CO stores during "rebreathing" allow separation of Vco and rate of metabolism of body CO The rebreathing technique prevents excretion of CO via the lung and therefore any change in the body stores should be determined by relative rates of endogenous production and metabolism (if no exchange occurs via skin) By adding a radioactive tracer $C^{14}O$ to the body stores the rate of loss of body CO can be estimated from measurements of the rate of decrease in blood radioactivity, from this measurement and the rate of change in body CO stores it is possible to calculate rate of production (6, 17) In Figure 4 results

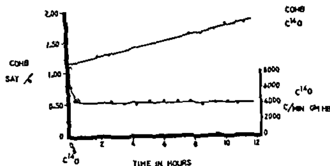


Fig 4 Changes in blood [COHb] and $C^{14}O$ during *a rebreathing experiment in* an anesthetized dog See text for description of experiment and discussion of results

of an experiment performed on an anesthetized dog are depicted (6, 17) $C^{14}O$ was introduced into the rebreathing system at time zero and was rapidly taken up *into pulmonary capillary* blood The initial decrease in radioactivity in venous blood, expressed per gram hemoglobin apparently is a result of intra vascular mixing and transport of the *tracer into* extravascular *CO stores It* can be shown that these processes occur at approximately the same rate (T 1/2 12 5 minutes) as mixing of chromium 51 labeled erythrocytes injected intra venously (6 17) Following this transient period of time the blood radioactivity decreased in an apparent linear manner for many hours This loss from the blood is thought to be a result of metabolic *consumption* of CO rather than diffusion of the tracer into an extremely slowly equilibrating extravascular CO pool since the rate of decrease was constant Furthermore it was shown that $C^{14}O$ is *oxidized to* $C^{14}O_2$ in these experiments There was a discrepancy, however between the average rate of loss of the tracer from blood in 8 experiments (0 9 % hr) and rates of oxidation to $C^{14}O_2$ (which averaged 0 3 %/hr) suggesting there is an additional process that is consuming CO Data from the present study suggest that this unexplained process is not skin transport of CO Comparison of the rates of con sumption of the tracer and increase in body CO stores suggests that the rate of production is approximately 20 times greater than the rate of metabolism in dogs at *normal* [COHb] The rates of disappearance of the tracer from the body and oxidation of the tracer to $C^{14}O_2$ did not vary with increases in [COHb] from normal levels of 0 9 % to over 35 % *saturation indicating that* metabolism of CO is a first order process and that the rate of $C^{14}O$ metabolism should increase as the body $C^{14}O$ stores increase Therefore at elevated blood *[COHb] the rate* of metabolism becomes a much more significant determinant of body CO stores than at normal [COHb] Measurements of loss of $C^{14}O$ from blood (with breathing in a closed system) have not been performed in man, however, in two experiments it was found that oxidation of $C^{14}O$ to $C^{14}O_2$ occurred at rates of 0 12 and 0 16 %/hr, (6,17) rates significantly less than found in dogs *It* would be estimated that the

18—663577 S pH 4

a 2 ml blood sample and transferring it into an evacuated ionization chamber The method can detect 1×10^{-4} μc

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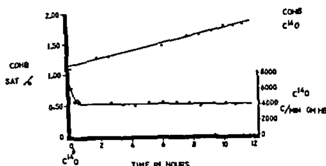


Fig 4 Changes in blood [COHb] and $C^{14}O$ during a rebreathing experiment in an anesthetized dog See text for description of experiment and discussion of results

of an experiment performed on an anesthetized dog are depicted (6, 17) $C^{14}O$ was introduced into the rebreathing system at time zero and was rapidly taken up into pulmonary capillary blood The initial decrease in radioactivity in venous blood, expressed per gram hemoglobin apparently is a result of intravascular mixing and transport of the tracer into extravascular CO stores It can be shown that these processes occur at approximately the same rate (T 1/2 12 5 minutes) as mixing of chromium 51 labeled erythrocytes injected intravenously (6 17) Following this transient period of time the blood radioactivity decreased in an apparent linear manner for many hours This loss from the blood is thought to be a result of metabolic consumption of CO rather than diffusion of the tracer into an extremely slowly equilibrating extravascular CO pool since the rate of decrease was constant Furthermore it was shown that $C^{14}O$ is oxidized to $C^{14}O_2$ in these experiments There was a discrepancy however, between the average rate of loss of the tracer from blood in 8 experiments 0 9 % hr) and rates of oxidation to $C^{14}O_2$ which averaged 0 3 %/hr) suggesting there is an additional process that is consuming CO Data from the present study suggest that this unexplained process is not skin transport of CO Comparison of the rates of consumption of the tracer and increase in body CO stores suggests that the rate of production is approximately 20 times greater than the rate of metabolism in dogs at normal [COHb] The rates of disappearance of the tracer from the body and oxidation of the tracer to $C^{14}O_2$ did not vary with increases in [COHb] from normal levels of 0 9 % to over 35 % saturation indicating that metabolism of CO is a first order process and that the rate of $C^{14}O$ metabolism should increase as the body $C^{14}O$ stores increase Therefore at elevated blood [COHb] the rate of metabolism becomes a much more significant determinant of body CO stores than at normal [COHb] Measurements of loss of $C^{14}O$ from blood (with breathing in a closed system) have not been performed in man, however in two experiments it was found that oxidation of $C^{14}O$ to $C^{14}O_2$ occurred at rates of 0 12 and 0 16 %/hr, (6 17) rates significantly less than found in dogs It would be estimated that the

a 2 ml blood sample and transferring it into an evacuated ionization chamber The method can detect 1×10^{-4} μc

Results

Skin CO Exchange Experiments

Results from an experiment where approximately 0 5 % CO was placed in contact with skin and mucous membranes of an anesthetized dog are shown in Figure 2 In two experiments the rate of increase of body CO stores did not change compared to the control, therefore, significant quantities of CO did not traverse the skin or mucous membranes The ratio of CO/Ne in box air did not change during the experiment and significant quantities of Ne were not found in the gas in the rebreathing system

The body CO stores increased markedly in all three experiments on human subjects indicating uptake of CO via the skin Data are shown in Figure 3 Skin uptake was apparently independent of external Pco over the range 100—700 mmHg At external Pco less than 100 mmHg skin uptake appeared to be proportional to external Pco In the experiment where CO was in contact with the skin of the right arm, CO uptake was significantly greater at equivalent external Pco when expressed in terms of ml/hr $\times$ m^2 than in the two experiments where 90 % of the body's skin was exposed to CO

Formate Injection Experiments

No radioactivity was measureable in the body CO stores up to 4 hours following injection of sodium formate-C^{14}

Discussion

Processes That Influence Body CO Stores and Blood Carboxyhemoglobin % Saturation

It seems obvious that the rate of endogenous CO production is a primary determinant of the body CO stores and blood carboxyhemoglobin % saturation, however, the other variables involved that influence the body CO stores and the interaction of these variables have rarely been considered The physiological variables that are thought to influence body CO (4, 6, 17) include Vco, rate of metabolism of body CO, the variables that determine excretion of CO in the lung, variables that influence uptake of CO from the environment, and variables that influence distribution of CO in the body In the present study we have investigated the possibility that skin CO transport or exchanges of body CO with body formate might also be significant variables

Studies of changes in the body CO stores during "rebreathing" allow separation of Vco and rate of metabolism of body CO The rebreathing technique prevents excretion of CO via the lung and therefore any change in the body stores should be determined by relative rates of endogenous production and metabolism (if no exchange occurs via skin) By adding a radioactive tracer $C^{14}O$ to the body stores the rate of loss of body CO can be estimated from measurements of the rate of decrease in blood radioactivity, from this measurement and the rate of change in body CO stores it is possible to calculate rate of production (6, 17) In Figure 4 results

[COHb] (4) Pco in pulmonary capillary blood is primarily a function of [COHb] and ($\bar{P}co_2$) Transport between blood and alveolus is a function of Dco and Pco gradients and the latter is a function of alveolar ventilation If one assumes even distribution of VA/Dco and VA and pulmonary capillary blood flow throughout the lung it is possible to describe the processes that influence excretion of endogenously produced CO in equation form for the steady state situation where Vco equals the rate of CO excretion (4)

$$[COHb] = \frac{Vco\ M\ [O_2Hb]}{\bar{P}co_2} \left[\frac{1}{Dco} + \frac{Pb\ P_{H_2O}}{VA}\right] \qquad (1)$$

where Vco is in ml/min M is an unitless equilibration constant for the reaction of CO with O_2Hb (10) Pb is barometric pressure P_{H_2O} is the vapor pressure for water [O_2Hb] is oxyhemoglobin % saturation This equation demonstrates the importance of Dco VA and $\bar{P}co$ as determinants of [COHb] and the body CO stores The applicability of Equation 1 to normal man has been supported by data obtained in experiments where $\bar{P}co$ was changed by having the subjects breathe 100 % O_2 (4) VA measured serially and the resultant change in [COHb] determined The time constant of the CO production excretion system is very much shorter at very high pulmonary capillary oxygen tensions than it is breathing air and it is possible to achieve an approximate steady state in 3 hours No exogenous CO was present in inspired air in this experiment so all of the body CO stores could be considered as endogenous CO It was demonstrated that it is possible to predict [COHb] with a relatively small error using Equation 1 in normal subjects who presumably had even pulmonary distribution of ventilation, Dco and capillary blood flow Since the [COHb] and body CO stores can be predicted with an equation that considers only production and excretion other variables probably have a relatively small effect on the CO stores It is also possible to calculate transients following changes in a determining variable (4) The time constant as indicated above, is very long breathing air (approximately 5 hours) and it can be shown that the time required to achieve a new steady state following a change in any of the variables in Equation 1 is over 24 hours VA and Dco have diurnal variations and it can be shown that variation in VA alone from nocturnal values of 4 liters/min to values of 8 liters/min during the day would result in a rate of excretion during the day approximately 2 × greater than that at night even though Vco is constant Although a steady state situation is hypothetical consideration of equations for a steady state illustrates the processes involved in determining [COHb]

It is worthwhile to note that the relationships of [COHb] and Vco in a steady state is a constant one if the other variables are kept constant This relationship was predictable on the basis of the work of Engstedt (11) who found a linear relationship between erythrocyte destruction and [COHb] in patients with hemolytic anemia Plots of Vco versus [COHb] in patients with hemolytic anemia (7) show scatter

rate of metabolism of CO in man, as well, would be very small compared to the rate of production Chemical studies have suggested that oxidation of CO occurs in mitochondria CO is thought to combine with reduced cytochrome oxidase and this complex in turn to react with oxidized cytochrome oxidase giving cytochrome oxidase and CO (24)

Since the rate of production of CO appears to be very large compared to the rate of metabolism of this gas it is possible to extimate Vco from the rate of increase of blood CO and body dilution of CO (1) As indicated above the extravascular CO stores appear to equilibrate with the vascular stores at about the same rate as intravascular mixing, therefore, changes in blood CO precisely reflect changes in the body CO stores Furthermore, evidence is available that partition of CO between blood and extravascular stores is constant over a wide range of [COHb] (6,17) Five or six venous blood samples are drawn for [COHb] analysis over a period of two hours while the subject is breathing in the closed system CO dilution is determined at the end of the experiment by adding a known quantity of CO to the body stores and measuring the resultant increase in [COHb] The method of determining [COHb] must be able to detect changes $\pm 0.02\%$ saturation The Rebreathing Method has been found to precisely measure simulated Vco in normal man and in anesthetized dogs (7) In normal man at rest Vco averages $0.42 \pm$ SD 0.07 ml/hr (1) For routine studies on man a rebreathing system has been designed (7) which does not require breathing through a mouthpiece and allows the Vco measurement to be performed on debilitated patients

Previously it has been thought that the blood [COHb] closely reflected Vco, (11), however, it appears now that blood CO is also influenced by many other variables as discussed above (4) and the method of estimating Vco from single [COHb] measurement has been discarded Vco has also been estimated from the rate of CO excretion via the lungs (22) It is unfortunate that Vco cannot be precisely measured simply by determining the CO concentration of inspired and expired air and ventilation (as is possible in the case of CO_2 production by the body) since it is virtually impossible to achieve a steady state between Vco and rate of excretion (4) With the Rebreathing Method it is possible to measure an increase in CO production within minutes after it has occurred, however, an equivalent change in the rate of CO excretion would not occur for over 24 hours (4) The development of the Rebreathing Method has allowed quantitative studies of the relationships of CO production and other variables that influence the body CO stores and also investigations of the relationships of hemoglobin and heme catabolism and Vco

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rate of metabolism of CO in man, as well, would be very small compared to the rate of production. Chemical studies have suggested that oxidation of CO occurs in mitochondria. CO is thought to combine with reduced cytochrome oxidase and this complex in turn to react with oxidized cytochrome oxidase giving cytochrome oxidase and CO_2 (24).

Since the rate of production of CO appears to be very large compared to the rate of metabolism of this gas it is possible to extimate Vco from the rate of increase of blood CO and body dilution of CO (1). As indicated above the extravascular CO stores appear to equilibrate with the vascular stores at about the same rate as intravascular mixing, therefore, changes in blood CO precisely reflect changes in the body CO stores. Furthermore, evidence is available that partition of CO between blood and extravascular stores is constant over a wide range of [COHb] (6,17). Five or six venous blood samples are drawn for [COHb] analysis over a period of two hours while the subject is breathing in the closed system. CO dilution is determined at the end of the experiment by adding a known quantity of CO to the body stores and measuring the resultant increase in [COHb]. The method of determining [COHb] must be able to detect changes $\pm$ 0.02 % saturation. The Rebreathing Method has been found to precisely measure simulated Vco in normal man and in anesthetized dogs (7). In normal man at rest Vco averages 0.42 $\pm$ SD 0.07 ml/hr (1). For routine studies on man a rebreathing system has been designed (7) which does not require breathing through a mouthpiece and allows the Vco measurement to be performed on debilitated patients.

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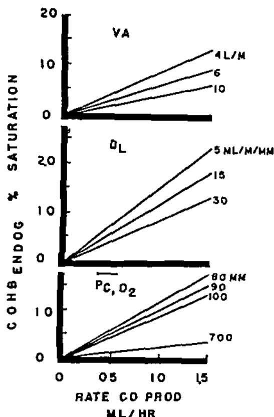


Fig 5 The effects of endogenous variables on blood carboxyhemogobin % saturation This figure demonstrates the effect of changes of alveolar ventilation (VA) diffusing capacity of the lung (D_L), and mean capillary O_2 tension ($\bar{P}co_2$) on blood carboxyhemoglobin concentration resulting from endogenous CO production for rates of CO production varying from zero to *1 5 ml per hour VA is 4 liters per minute,* D_L is 30 ml per minute per mmHg and $\bar{P}co_2$ is 100 mm Hg unless otherwise noted Reproduced with the permission of *The Journal of Clinical Investigation* (4)

Exogenous CO is absorbed via the lung and contributes to the body CO *stores and blood [COHb] The air CO* concentration in our laboratories and wards in the Hospital of the University of Pennsylvania varied over a period of several months from 4×10^{-5} to $4 \times$ *10^{-4} % averaging 2 2 × 10^{-4} % It is* estimated (4) that in the absence of endogenous CO production that the presence of 2 2 × 10^{-4} % CO in inspired air would cause a [COHb] of approximately 0 5% This would account for slightly over half of the blood [COHb] found in normal subjects in Philadelphia It can be calculated from Equation 1 *that endogenous CO should result in* [COHb] of approximately 0 4 % saturation in a normal human *The sum of* the calculated exogenous and endogenous [COHb] is similar to the average normal

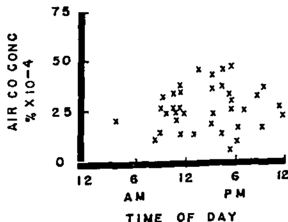


Fig 6 CO concentrations in air This figure illustrates the variation in air CO % that occurred over a period of several months The time of air sampling is indicated on abscissa *These* measurements were made with an infrared CO meter Reproduced with the permission of *The Journal of Clinical Investigation (4)*

measured [COHb] of 0 88 % saturation (2) *From air CO concentration* measurements published by Sjostrand (22) the ambient concentration of this gas in the late 1940s was considerably less in Sweden than found in Philadelphia at the present time This probably explains the lower normal [COHb] reported for normal subjects living in Stockholm CO measurements in Philadelphia air are illustrated in Figure 6

Processes that influence endogenous production of CO

The concept that CO originates from a heme bridge carbon atom during hemo globin catabolism seems well founded at the present time

The pioneering studies of Sjostrand have been mentioned (21, 22, 23) Recently the concept was evaluated in experiments where the heme bridge carbon atoms were labeled with C^{14} (8) It was demonstrated that $C^{14}O$ was produced in anesthetized dogs following intravenous administration of (a) damag ed erythrocytes containing radioactive hemoglobin (b) dialyzed C^{14} hemo globin solutions and (c) solutions of reconstituted hemoglobin containing C^{14} hemin and unlabeled globin $C^{14}O$ was not produced when solutions of re constituted hemoglobin containing un labeled hemin and labeled globin were administered Although chemical oxida tion of hemoglobin results in random *splitting of the heme molecule* at all four bridge carbon atoms (18) physiolog ically this occurs only at the alpha po sition (12) so presumably CO originates from the heme alpha methene bridge carbon atom The CO specific activities found in the above experiments were consistent with this concept. CO molar yields in experiments where damaged erythrocytes are injected (3) or experiments where hemoglobin solutions are administered (8) are very nearly 100 %, CO presumably is produced in the reticuloendothelial system although there is as of yet no direct evidence in this regard. The processes that influence rate of CO production arising from hemoglobin catabolism, therefore, appear to be those that determine the rate of erythrocyte catabolism and possibly variables that influence the molar yield of CO

There is evidence that there is a source or sources of endogenous CO in normal man other than catabolism of hemoglobin in senescent erythrocytes Calculations of hemoglobin turnover and measurement of CO production indicate that about 25 % of the normal rate of CO *production of 0 42 ml/hr cannot be* explained on the basis of catabolism of

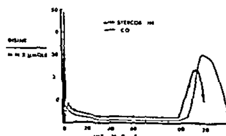


Fig 7 CO and stercobilin radioactivity following administration of glycine $2C^{14}$ to a normal male subject (25) One hundred μc of the isotope were administered on day zero Specific activity of body CO was corrected for the presence of exogenous CO in body stores Stercobilin radioactivity is expressed in terms of one heme glycine" carbon atom so that it is comparable with CO specific activity

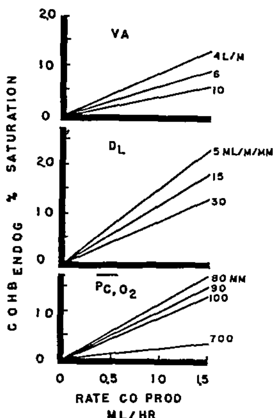


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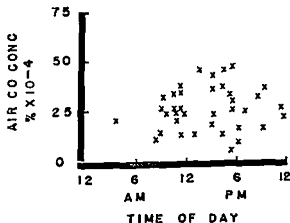


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Processes that influence endogenous production of CO

The concept that CO originates from a heme bridge carbon atom during hemoglobin catabolism seems well founded at the present time

The pioneering studies of Sjostrand have been mentioned (21 22, 23) Recently the concept was evaluated in experiments where the heme bridge carbon atoms were labeled with C^{14} (8) It was demonstrated that $C^{14}O$ was produced in anesthetized dogs following intravenous administration of (a) damaged erythrocytes containing radioactive hemoglobin, (b) dialyzed C^{14} hemoglobin solutions and (c) solutions of reconstituted hemoglobin containing C^{14}-hemin and unlabeled globin $C^{14}O$ was not produced when solutions of reconstituted hemoglobin containing unlabeled hemin and labeled globin were administered Although chemical oxidation of hemoglobin results in random splitting of the heme molecule at all four bridge carbon atoms (18) physiologically this occurs only at the alpha position (12) so presumably CO originates from the heme alpha methene bridge carbon atom The CO specific activities found in the above experiments were consistent with this concept CO molar yields in experiments where damaged erythrocytes are injected (3) or experiments where hemoglobin solutions are administered (8) are very nearly 100 %, CO presumably is produced in the reticuloendothelial system although there is as of yet no direct evidence in this regard The processes that influence rate of CO production arising from hemoglobin catabolism, therefore, appear to be those that determine the rate of erythrocyte catabolism and possibly variables that influence the molar yield of CO

There is evidence that there is a source or sources of endogenous CO in normal man *other than catabolism of hemoglobin* in senescent erythrocytes Calculations of hemoglobin turnover and measurement of CO production indicate that about 25 % of the normal rate of CO production of 0 42 ml/hr cannot be explained on the basis of catabolism of

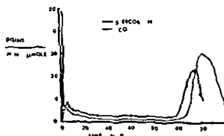


Fig 7 CO and stercobilin radioactivity following administration of glycine 2C 14 to a normal male subject (25) One hundred μc of the isotope were administered on day zero Specific activity of body CO was corrected for the presence of exogenous CO in body stores Stercobilin radioactivity is expressed in terms of one heme glycine carbon atom so that it is comparable with CO specific activity

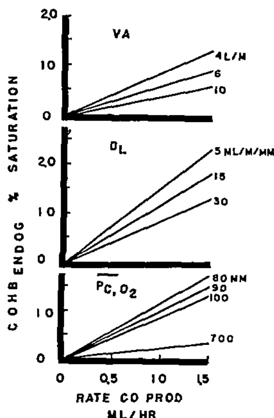


Fig 5 The effects of endogenous variables on blood carboxyhemogobin % saturation This figure demonstrates the effect of changes of alveolar ventilation (VA), diffusing capacity of the lung (D_L), and mean capillary O_2 tension ($\overline{P}co_2$) on blood carboxyhemoglobin concentration resulting from endogenous CO production for rates of CO production varying from zero to 1 5 ml per hour VA is 4 liters per minute, D_L is 30 ml per minute per mmHg, and $\overline{P}co_2$ is 100 mm Hg unless otherwise noted Reproduced with the permission of *The Journal of Clinical Investigation* (4)

probably resulting from patient to patient variations in Dco, VA, $\overline{P}co_2$ and exposure to environmental CO, however, this data also suggests a linear relationship of Vco and [COHb] Figure 5 demonstrates effect of several *excretory* variables on steady state [COHb]

Exogenous CO is absorbed via the lung and contributes to the body CO stores and blood [COHb] The air CO concentration in our laboratories and wards in the Hospital of the University of Pennsylvania varied over a period of several months from 4×10^{-5} to 4×10^{-4} % averaging $2 2 \times 10^{-4}$ % It is estimated (4) that in the absence of endogenous CO production that the presence of $2 2 \times 10^{-4}$ % CO in inspired air would cause a [COHb] of approximately 0 5% This would account for slightly over half of the blood [COHb] found in normal subjects in Philadelphia It can be calculated from Equation 1 *that endogenous CO should result in* [COHb] of approximately 0 4 % saturation in a normal human The sum of the calculated exogenous and endogenous [COHb] is similar to the average normal

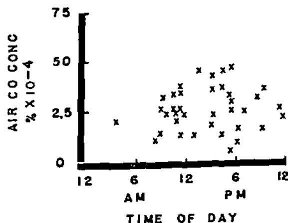


Fig 6 CO concentrations in air This figure illustrates the variation in air CO % that occurred over a period of several months The time of air sampling is indicated on abscissa These measurements were made with an infrared CO meter Reproduced with the permission of *The Journal of Clinical Investigation* (4)

icantly contributes to endogenous CO production At the present time it has been demonstrated that CO is endogenously produced in the dog (8), sheep (16) and rat (14) as well as man The anesthetized dog preparation appears to be particularly useful in studies of CO production since damaged erythrocyte injection experiments give identical results in dogs as in normal man

In Figure 8 are summarized the known processes that determine body CO stores and blood [COHb]

Skin CO Exchange

It seemed rather unlikely that skin CO exchange would significantly influence the body CO stores of an experimental subject not respiring in a rebreathing system since the CO diffusing capacity of the lung should be several hundred times greater than that of skin on the basis or relative thickness of skin and the alveolar membrane and greater diffusing area of the lungs The tracer studies described above, however, indicated that $C^{14}O$ was lost from the blood of an anesthetized dog breathing in a closed system at a greater rate than could be explained on the basis of oxidation of $C^{14}O$ to $C^{14}O_2$ and it was possible that the tracer might be lost via the skin and mucous membranes In the experiments on dogs CO at a tension of approximately 3 6 mm Hg placed in the box in contact with skin and mucous membranes however CO uptake did not occur at significant rates as evidence by the finding that the rate of increase of body stores did not change The normal transdermal CO gradient can be estimated from the average air CO concentration (approximately $1\ 5 \times 10^{-2}$ mmHg) and skin capillary Pco This latter was calculated from the Haldane Equation (10) (assuming a mean capillary PO_2 of 50 mmHg) to be 3×10^{-3} mmHg giving a calculated normal skin gradient of $1\ 5 \times 10^{-3}$ mmHg Since no skin uptake occurred at a gradient approximately 2 400 times greater than normal, it is extremely unlikely that skin CO exchange influences the body CO stores

In man it was possible to measure CO uptake by skin when very high concentrations of CO were placed in contact with skin Gradients of $2\ 5 \times 10^{3}$ to $5\ 0 \times 10^{4}$ orders of magnitude greater than the normal transdermal gradient were obtained These data as shown in Figure 3, are particularly interesting since CO uptake appeared not to vary as a function of external Pco over range 100—700 mmHg, although at external Pco of less than 100 mmHg CO uptake appeared to be proportioned to external Pco Similar results have been found in studies of CO uptake in the urinary bladder (5) and ileum (19), and it has been postulated (5) that CO uptake over the high Pco range where uptake was independent of Pco gradient is limited by blood flow through superficial capillaries It can be shown mathematically that CO uptake into a capillary ur rounded by a tissue cylinder remains diffusion limited until CO uptake versus rate blood flow is sufficiently great to saturate end-capillary hemoglobin (5) This concept appears to explain CO uptake in hollow organs where CO flux is proportional to lumen Pco at low Pco and therefore diffusion limited and independent of lumen Pco at high lumen

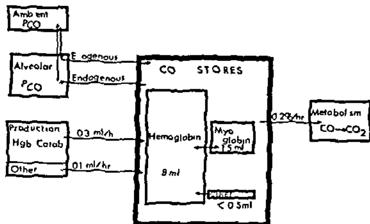


Fig 8 Schematic representation of variables that influence body CO stores This figure summarizes current concepts regarding variables that influence body CO stores

hemoglobin in circulating erythrocytes (1, 3) In addition administration of glycine 2C[14] to a normal subject resulted in the appearance of radioactivity in the body CO stores on days 1—10 as well as the late peak from 100—130 days accountable on basis of catabolism of erythrocyte hemoglobin (25) Results from such an experiment are shown in Figure 7 (25) The area under the early peak is approximately 20 % of the area of the late peak as would be expected if the early peak conformed to the "excess" CO production mentioned above Of course, the early peak of radioactivity in bile pigment following glycine-2C[14] has been known for some time (15), and was also found in the experiment depicted in Figure 7 Similar experiments have recently been performed on rats (14) Evidence is accumulating that CO and bile pigment are produced in parallel as a result of processes contributing to the "early" peak as well as late hemoglobin peak (14, 25, 26) The process or processes that contribute to the "early peak" of bile pigment and CO production are not completely clear, however, very recent experiments by Schmid and coworkers (20) demonstrate that induction of liver microsome by administration of phenobarbital results in an increase in the "early" peak of bile pigment radioactivity following administration of radioactive glycine to rats Recent unpublished experiments performed in our laboratory have shown there is a significant increase in V_{CO} in normal subjects following phenobarbital administration (9) These experiments suggest that catabolism of liver microsomal heme enzymes, more specifically cytochrome p-450, may be the origin of some or most of the "excess" CO fraction The possibility that CO and bile pigment are produced as a by-product of protoporphyrin catabolism has not been excluded $C^{14}O$ is produced following intravenous administration of C[14] protoporphyrin (8), however, radioactive hemin was found in the liver of one of the experimental animals suggesting that the chemical reaction of protoporphyrin to CO involves liver hemin The evidence that ineffective erythropoiesis contributes to this CO fraction in normal man has been reviewed (25) There is no direct evidence that bacterial metabolism signif

following intravenous injection of sodium formate C^{14} suggesting that the body CO and formate pools do not exchange to a significant degree

4 Recent concepts regarding processes that influence the body CO stores and endogenous CO production were discussed

Acknowledgements

The author would like to acknowledge the constant stimulation and support of Dr Robert E. Forster and the expert technical assistance of Mr Robert Ersek and Mrs. Beverly Florey

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Pco (blood flow limited) If this is also true in the case of skin CO uptake it would appear that the separation between diffusion limited and blood flow limited CO uptake might occur at external Pco of approximately 100 mm Hg

If CO uptake is diffusion limited at low external Pco the uptake rates can be extrapolated to normal skin CO gradients Extrapolation of data in experiments 4-4 62 and 5-11-62 to a normal Pco gradient of 1 5 $\times$ 10^{-3} mm Hg indicates that calculated CO uptake at that gradient would be approximately 1 $\times$ 10^{-4} ml/hr $\times$ m^2 and suggests that in man, as well, skin CO exchange is of no significance as a determinant of body CO stores

CO uptake studies may be useful in investigations of skin blood flow In our experiments skin and body temperature were not monitored or controlled In the whole body experiments the subjects complained of feeling cold since they were unclothed at room temperature The greater CO uptake in the experiment using only the arm may be a result of temperature or a larger cutaneous blood flow in arm skin

Blood flow can be calculated from CO uptake measurements in a blood flow limited situation as below (5)

$$Q = \frac{\text{Rate CO uptake}}{[Hb] \times 1\ 34} \quad (2)$$

Q is rate blood flow in ml/min, [Hb] is blood hemoglobin concentration in gm/ml, and 1 34 is the CO capacity of blood in ml CO per gm Hb The calculated blood flow from experiment 4-26 62 was approximately 100 ml/hr $\times$ m^2, for 4-4 62 and 5-11-62, blood flows were 53 and 33 ml/hr $\times$ m^2 respectively These values are considerably less than published rates of skin blood flow measured plethysmographically or thermally (13) which were made at normal or elevated skin temperatures We have been unable to find estimates in the literature of skin blood flow made in shivering man and it is possible that skin blood flow actually is diminished to the extent suggested by our calculations It is also possible that the CO gas exchange vessels of the skin represent only a portion of the total skin capillary bed

Formate Injection Experiments

From the 2 experiments in which C^{14} formate was administered to anesthetized dogs and no measurable radioactivity found in the body CO stores, it can be said that less than 0 001 % of the injected radioactivity exchange into the CO stores This tends to exclude this pathway as important in determining body CO stores

Summary

1 Evidence is presented that transdermal CO exchange in man and dog is not a significant determinant of body CO stores under normal conditions

2 CO uptake via the skin in man was independent of the skin CO gradient when external *Pco* was greater than *100* mmHg suggesting that CO uptake is blood flow limited over this gradient range At lower external Pco the CO uptake appeared to be at least partially diffusion limited

3 No radioactivity was found in the body CO stores within four hours

following intravenous injection of sodium formate C^{14} suggesting that the body CO and formate pools do not exchange to a significant degree

4 Recent concepts regarding processes that influence the body CO stores and endogenous CO production were discussed

Acknowledgements

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From the Clinical Laboratory and the Department of Anaesthesiology S t Eriks Sjukhus Stockholm
and the Clinical Laboratory Sahlgrenska Sjukhuset, Goteborg

Parenteral nutrition by a solution of crystalline amino acids

By

PETER FURST, BO HALLGREN, BERTIL JOSEPHSON and ERIK VINNARS

It is a common experience that solutions of hydrolyzed or digested casein administered intravenously to patients for amino acid nutrition as first suggested by Elman & Weiner (4), may incidentally cause nausea vomiting and other discomforts Such symptoms can be provoked in almost any subject by increasing the volume or rate of the hydrolysate infusion It does not seem probable that the amino acids are responsible for these symptoms even if some of them may be toxic when given in large amount Solutions of pure 1 amino acids have therefore been suggested for parenteral nitrogen nutrition with the intention of avoiding disagreeable side-effects Since the essential amino acids have recently become available at a reasonable price some of these solutions are now on the market However it is not yet established that the composition of these solutions is optimal with respect to the nitrogen balance in man For this reason we have studied some amino acid patterns in an attempt to suggest a solution giving a positive nitrogen balance without causing discomfort or other side-effects to the patients

After several attempts not reported here we have arrived at a composition which is a modification of a solution suggested by Bansi et al (2), the main difference being that the concentration of essential amino acids in our solution is about twice that of Bansi et al where as the amount and type of non-essential nitrogen i e 35 g glycine per litre is the same in both solutions Our solution has an E/T value (milligrams of the essential amino acids per g of total nitrogen) of 2 6 (6)

This paper reports the results on healthy volunteers the results on clinical cases after surgery will be reported elsewhere In the reported experiments the metabolic effect of the amino acid solution was compared with a commercial solution made by dialysis of digested casein having an E/T value of 3 0 In this solution about two thirds of the casein has been digested to individual amino acids whereas the remainder consists of dialysable peptides (18)

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TABLE II Hospital nitrogen poor food daily ration

Type	Weight g	N %	N g/day
White soft bread	22 2	1 47	0 33
White crisp bread	12 7	1 60	0 20
Rice pancake	80 —	0 69	0 55
Celery croquette	64 —	0 51	0 33
Mushroom sauce	96 —	0 53	0 51
Lingonberries	15 7	0 06	0 01
Orange marmalade	34 1	0 03	0 01
Apple	100	0 04	0 04
Potatoes	75 —	0 33	0 25
		Sum	2 23

Calories 2240

venously over a period of 4 hours, and on the 12th and 13th day one litre of the digested casein solution The experiment was concluded by two days (the 14th and 15th) on the standard diet only

Group 2 (Fig 2) Exactly the same experiment as in group 1 was carried out on two men

Group 3 (Fig 3) The corresponding experiment as in group 1 was carried out on two women except that the digested casein solution was given on the 8th and 9th day and the amino acid solution on the 12th and 13th day

Group 4 Two 20 year old women received a diet similar to that used in the previous groups but containing more calories and nitrogen (3 6 g per day) than the diet recorded in Table II The diet was given for 4 days only One of the subjects had an infusion of 1 litre of the amino acid solution on the 5th and the 6th day of the experiment but in contrast to the subjects in group 1, 2 and 3, no food was given on the days of infusion Instead she received, in addition to the amino acids, 500 ml of a commercial, nitrogen free fat-emulsion and 1000 ml of a 20 per cent fructose solution intravenously equivalent to 2000 calories On the 7th day she again ate only the original diet In this case no comparison with the digested casein was made The results from this subject are recorded in Fig 4 The other subject in this group discontinued the experiment after the first day of infusion The result turned out very similar to the other experiment in this group

The intravenous infusions were given under constant supervision in the hospital They were started about 8 o clock in the morning and lasted about 4 hours Venous plasma samples were taken before the start of the diet immediately before during and immediately after the infusions

All 24 hour urine samples were analysed for total nitrogen (micro-Kjeldahl) urea (3) creatinine (9) ammonia (3) α amino nitrogen (15), and pH Most of the urine samples were analysed for the individual amino acids by chromatography according to Moore and Stein The faeces was analysed for total nitrogen only (micro-Kjeldahl) The plasma samples were analysed for ammonia only (3)

Except for the time of infusion, the volunteers pursued their normal routine during the period of the experiment None of the subjects experienced any side-effects except for the fact that they considered the food monotonous and that some of them felt hungry

TABLE I Composition of the solutions used for parenteral nitrogen nutrition

	According to manufacturer*	According to analysis: Our solution*	According to analysis: Digested casein
l Threonine g/lit	2 0	2 0	3 9
l Valine g/lit	3 2	3 3	4 7
l Methionine g/lit	4 4	4 5	4 0
l Isoleucine g/lit	2 8	2 9	6 5
l-Leucine g/lit	4 4	4 4	10 2
l Phenylalanine g/lit	4 4	4 4	6 2
l Tryptophane g/lit	1 0	1 0	1 0
l Lysine	3 2	3 3	6 6
l Histidine g/lit	2 2	2 2	2 3
l Arginine g/lit	4 5	4 5	2 6
Glycine g/lit	34 0	35 0	2 0
Total nitrogen g/lit		10 5	14 3
α amino nitrogen g/lit		9 3	8 6
Ammonia nitrogen mg/lit		0 2—0 8	15—85
Sorbitol g/lit	50 0	50 0	—
Calories per lit		200	—
pH		5 0	5 0

* This solution (ASTRA 1738) has been made available by ASTRA, Sodetalje, Sweden

Experimental

Table I records the composition of the solution for parenteral amino acid nutrition used in all the experiments One litre of the solution contains the eight essential amino acids in concentrations corresponding to about four times the quantities given by Rose (13) as the minimum requirements for man The solution also contains the semi essential amino acids, histidine and arginine, and — for provision of non essential nitrogen — 35 g glycine per litre For increase of the caloric value 50 g sorbitol per litre was added, as suggested by Bansi et al (2)

Table I also records the composition of the commercial solution of digested and dialysed casein used for comparison The bulk concentration of α amino groups is very similar in both solutions

The experiments were carried out on nine reliable, adult healthy subjects, divided into four groups

Group 1 (Fig 1) Three women had nothing to eat for 15 consecutive days except the standard diet recorded in Table II Water *ad libidem* This diet corresponded to 2 23 g nitrogen and 2240 calories per day On the 8th and 9th day of the experiment one litre of the amino acid solution was given intra-

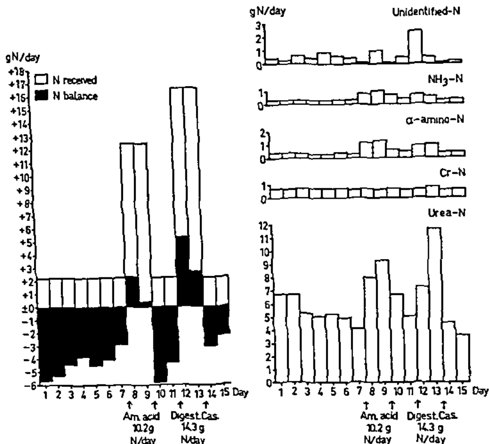


Fig 2 The same experiment as in Fig 1 carried out on two healthy male volunteers 34 and 44 years old

ments subjected to chromatography The mean values for plasma ammonia from all the subjects in groups 1 2 and 3 are recorded in Table III

Discussion

The experiments in the groups 1 and 3 differed only with respect to the order in which the two test solutions (amino acid solution and casein hydrolysate) were given The experiments in group 2 were identical with those in group 1 except for the fact that group 1 consisted of women and group 2 of men As expected the results from all these groups were similar except for the magnitude of the nitrogen balance During the days when only the standard food was given this balance was more negative in the men *than in the women and during the days* of infusion it was less positive This is entirely in accordance with the fact that men have a greater requirement than women for nitrogen and calories (17) For these reasons these three groups are discussed together The fact that the 24

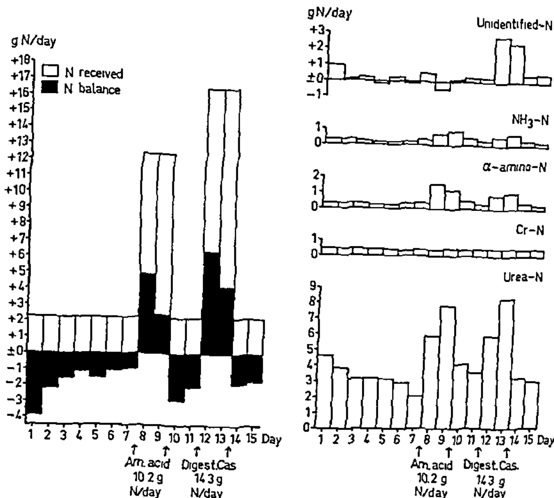


Fig 1 The effect on the nitrogen balance (left) and on the urine excretion of nitrogenous substances (*right*) by the amino acid solution and by the solution of digested and dialysed casein described in table I During the entire 15 days of the experiment including the days of *infusion* the subjects had nothing to eat except the standard diet described in table I On the 8th and 9th day 1 litre of the amino acid solution was infused intravenously for *a period of about 4 hours On the 12th and 13th day 1 litre of the solution of digested casein was administered* in the same way The diagram records the mean values from identical experiments on three healthy female volunteers aged 31—48 years

Results

The metabolic results are reported only in the figures and only as mean values from each group (except for group 4) since the results were surprisingly similar in all experiments in the individual groups Only the daily nitrogen loss from the faeces varied considerably and without any correlation to the diet and the infusions As this loss was of little influence on the nitrogen balance, it is not recorded The same experience has been reported previously (14) Since the amino acid pattern of the urine was not examined in all the cases no mean values have been calculated Instead the results from one of the subjects in group 1 are illustrated in Fig 5 Also these patterns turned out to be unexpectedly similar in all experi-

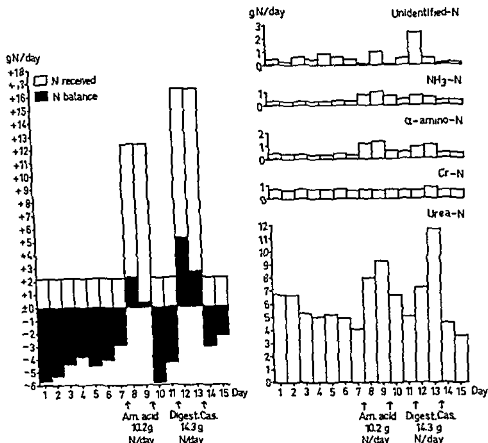


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The experiments in the groups 1 and 3 differed only with respect to the order in which the two test solutions (amino acid solution and casein hydrolysate) were given The experiments in group 2 were identical with those in group 1 except for the fact that group 1 consisted of women and group 2 of men As expected, the results from all these groups were similar except for the magnitude of the nitrogen balance During the days when only the standard food was given this balance was more negative in the men than in the women and during the days of infusion it was less positive This is entirely in accordance with the fact that men have a greater requirement than women for nitrogen and calories (17) For these reasons these three groups are discussed together The fact that the 24

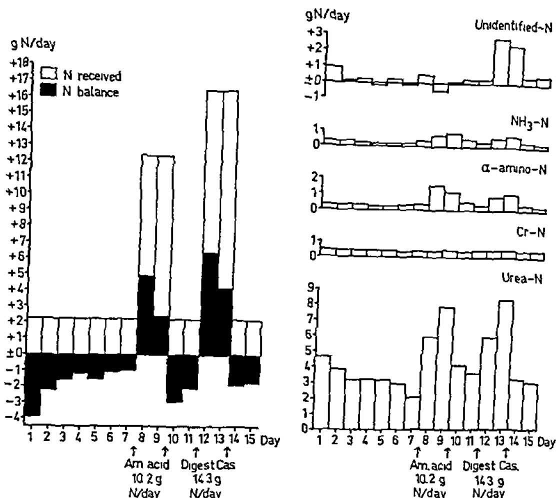


Fig 1 The effect on the nitrogen balance (left) and on the urine excretion of nitrogenous substances (right) by the amino acid solution and by the solution of digested and dialysed casein *described in table I* During the entire 15 days of the experiment including the days of infusion the subjects had nothing to eat except the standard diet described in table I On the 8th and 9th day 1 litre of the amino acid solution was infused intravenously for *a period of about 4 hours* On the 12th and 13th day 1 litre of the solution of digested casein was administered in the same way The diagram records the mean values from identical experiments on three healthy female volunteers, aged 31—48 years

Results

The metabolic results are reported only in the figures and only as mean values from each group (except for group 4), since the results were surprisingly similar in all experiments in the individual groups Only the daily nitrogen loss from the faeces varied considerably and without any correlation to the diet and the infusions As this loss was of little influence on the nitrogen balance, it is not recorded The same experience has been reported previously (14) Since the amino acid pattern of the urine was not examined in all the cases, no mean values have been calculated Instead the results from one of the subjects in group 1 are illustrated in Fig 5 Also these patterns turned out to be unexpectedly similar in all experi-

TABLE III Plasma ammonia in 7 healthy subjects before and after parenteral nitrogen administration ng/ml

	Amino acid sol		Digested casein sol	
	1st inf	2nd inf	1st inf	2nd inf
Before	0 69	0 82	0 65	0 80
After	0 84	1 05	1 15	1 33
Difference*	0 15	0 23	0 50	0 53

* Both the differences 0 50—0 15 and 0 53—0 23 are statistically highly significant (p ~ 0 001)

tained on the standard diet for a longer period before the infusions (7)

In all the experiments the nitrogen balance became positive when one litre of the nutrition solution was added by infusion to the constant oral supply of 2 2 g nitrogen per day in the standard diet Invariably the nitrogen balance was more positive on the first day of parenteral nutrition than on the second day The most probable explanation of this observation is that the main part of the nitrogen deficit is filled up already by the first day s infusion

In all three groups the nitrogen balance became more positive when the digested casein was given than after the infusion of the amino acid solution even if counted in per cent of the infused amount of nitrogen However the difference between the two solutions with respect to the nitrogen balance was not great enough to be significant for the nutrition

As will be demonstrated in another paper soon to be published the dif

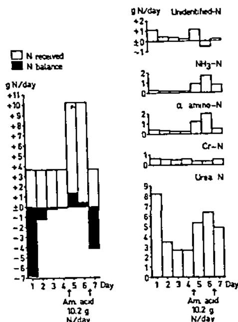


Fig 4 The effect of intravenous infusion of 1 litre of the amino acid solution on the nitrogen balance and excretion in one healthy 20 year old female volunteer who had nothing to eat except a standard food containing 3 8 g of nitrogen a day for 4 days prior to the infusion but (in contrast to the experiments in Fig 1—3) nothing to eat during the day of infusion In this experiment no digested casein was given

ference was the opposite after surgical operations In these cases the nitrogen balance was more advantageous to the patients when the amino acid solution was given This discrepancy indicates that the amino acid requirements may be different in health and after a major trauma e g operation In the normal cases the infusion had to fill up a deficit of mainly labile body protein whereas the surgical cases must be losing considerable quantities of endogenous cell protein

The results from this investigation differ from those of Bansi et al (2) in that a positive nitrogen balance was invariab

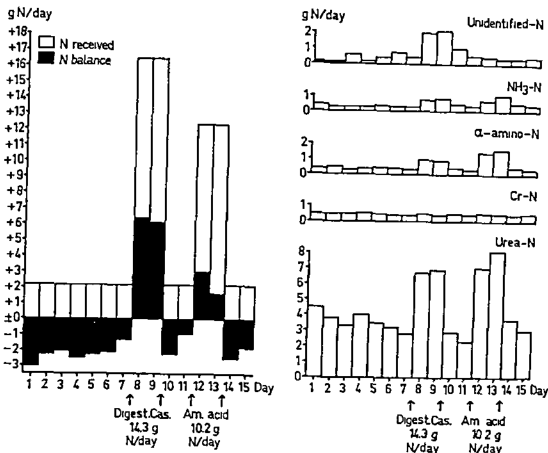


Fig 3 The corresponding experiment as in Fig 1 carried out on two healthy female volunteers 32 and 33 years of age, differing from the experiments in Fig 1 in that the order of infusions was reversed The infusion of digested casein was given on the 8th and 9th day, and the amino acid solution on the 12th and 13th day

hours' creatinine excretion was remarkably constant in each experiment is a check on the quantitative saving of the urine

The standard food was prepared to give an insufficient supply of nitrogen, since it was expected that the nutritive effect of the solution on trial would be better evaluated by experiments on subjects in nitrogen deficit rather than in subjects with a positive nitrogen balance Probably, a still more nitrogen deficient diet would have been advantageous, but it was considered that this diet was the lower limit at which reliable subjects would volunteer With respect to calories, the nutritive value of the standard food was considered satisfactory, which is of importance for optimal utilisation of the nitrogen

During the first days of the insufficient diet the nitrogen balance became considerably negative, with a tendency to approach a less negative but more stable level after a few days This is in accordance with previous observations of the so called 'labile body protein' first leaving the body during protein starvation, followed by a lower excretion of the endogenous nitrogen (1, 6) The results would probably have been more clearcut if the subjects had been main

the urine was almost identical in groups 1, 2 and 3. Whether the amino acids or the digested casein was given, the urea output increased by 3 to 4 g of nitrogen on the days of infusion. Without N[15] studies it is impossible to decide which amino acid it was that provided amino groups for this extra urea. According to the results of Sprinson and Rittenberg (16) it would seem probable that the main source was the high proportion of glycine in the amino acid solution. However, this explanation cannot be valid for the digested casein since the same urea increase was observed when the casein solution with its low glycine content was given.

The changes in urea output were almost a mirror image of the corresponding changes of the nitrogen balance. The balance was always more positive on the first day of infusion than on the second day. In contrast to this, the wasting of urea was always higher on the second day of infusion. Thus, when the amino acid deficit starts being saturated, the excess amino acids are wasted as urea.

The excretion of α-amino nitrogen was always increased by about 1 g per day whichever solution was given. This is in accordance with the augmented amino acid excretion demonstrated in Fig 3.

Also the ammonia excretion was a little higher during the days of infusion. The *most probable reason is that the* urine became a little more acid during the infusions. The increase in the excretion of amino acids and ammonia was of little influence on the nitrogen balance.

The quantity of unidentified nitrogen should always be slightly positive, since it represents the difference between total urine nitrogen, determined by the Kjeldahl method, and the sum of the nitrogen recovered as urea, creatinine, α-amino groups, and ammonia. It thus includes nitrogen from uric acid, creatine, purines etc. in the urine. The fact that this quantity was very low during the days on only the standard food may be considered as a check on the respective analyses.

It should be observed that the unidentified urine nitrogen was always increased when the digested casein was given, but not after the amino acid solution. As will be demonstrated in another paper, this extra unidentified nitrogen increased to a greater extent when the hydrolysate was administered to recently operated patients. It has been demonstrated that this nitrogen consists of peptides since hydrolysis of this fraction deliberates a corresponding amount of α-amino nitrogen (11). It has been pretended (10) that the digested casein solution should not exert any disagreeable side-effects. However, some of our operated patients (but none of the healthy volunteers) felt nauseated or vomited on receiving 1 litre of the digested casein, whereas the amino acid solution did not cause any discomfort. It seems probable that the peptides were responsible for these side effects.

The Moore and Stein analyses of the amino acid pattern of the urine during the days of infusion revealed that about *10 per cent of the* administered glycine was wasted irrespective whether the amino acids including 35 g of glycine or the *hydrolysate with 2 g of* this com

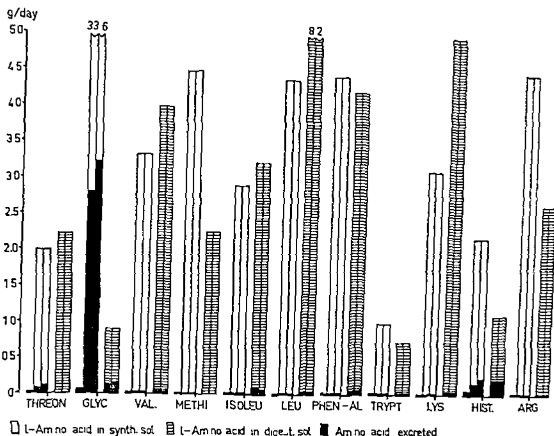


Fig 5 The quantities of administered amino acids and the Moore and Stein pattern of amino acids in the urine from the 2 days of amino acid infusion and the 2 days of infusion of digested and dialysed casein in one of the female volunteers recorded in Fig 1

ly found when the amino acid solution was administered whereas Bansi et al always observed a negative balance with their solution There were three main differences between the experiments of Bansi et al and those presented here 1) The amino acid solution of Bansi et al had the same source and amount of non-essential nitrogen as ours, i e 35 g of glycine per litre but only half the concentration of the essential amino acids This may be one reason for a less complete accumulation of nitrogen 2) The subjects in the Bansi experiments were patients with gastric ulcers or gastritis, who may have had a more labile nitrogen balance than our healthy volunteers 3) During the days of infusion Bansi et al did not give their subjects any food, whereas our volunteers ate the same standard diet as during the days preceding the infusions Consequently it could not be ruled out that the essential amino acids provided with the food protein may have rendered the nitrogen balance positive in our experiments In order to examine the influence of the food on the results the group 4 experiments were carried out Even if no food was given our solution turned the negative nitrogen balance into a positive one, although less positive than in the other experiments (Fig 4)

The pattern of nitrogen excretion in

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Reprints from professor Bertil Josephson Clinical Central Laboratory St Erik s Hospital, Stockholm

pound were given Of the other amino acids, only histidine appeared in quantities of any significance The histidine excretion had been previously observed by Bansi and coworkers (2) who related it to the limited capacity of the renal tubules to reabsorb this amino acid The fact that also threonine had a slight tendency to be increased in the urine when the amino acids were infused, may depend on competition by the glycine for tubular *reabsorption in the kidneys (8)*

As demonstrated in Table III, the blood ammonia concentration increased slightly by infusion of both the solutions, the increase during administration of the digested casein being significantly higher than when the amino acid solution was given However, also during infusion of the hydrolysate the ammonia concentration was well below the level at which subjective symptoms of ammonia intoxication appear Thus the disagreeable symptoms sometimes appearing during such an infusion can scarcely be due to an increase of the blood ammonia The observation that an amino acid infusion increased the blood ammonia content less than an infusion of casein hydrolysate may depend on the higher amount of arginine in the former solution It has been found (12) that arginine may capture ammonia, bringing it into the Krebs Henseleit cycle This explanation may be valid, even if arginine has no effect on the increase of ammonia in cases of liver insufficiency (5)

In contrast to published reports that glycine may exert toxic influences (12), we have not observed any side effects from the amino acid solution On the contrary, we can confirm previous observations (2, 7) that glycine is a possible way to supply non essential nitrogen, provided that the essential amino acids and arginine are administered in sufficient quantities

In papers, soon to be published, the effect of the amino acid solution on operated, surgical cases will be reported We will also report the effect of other solutions, including ammonium salts, *on the nitrogen balance in human* subjects

Summary

A solution containing the essential amino acids and histidine and arginine, and — as a source of non essential nitrogen — a sufficient amount of glycine is suggested for intravenous nitrogen nutrition in man The nutritional effect of this solution was tested on 9 healthy volunteers In all the subjects a negative nitrogen balance, due to nitrogen starvation turned positive when 1 litre of the solution was given per day No unpleasant side effects were observed

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patient, a 33 year old white man are presented elsewhere (30) The phenomenon has also been demonstrated in his sister who has been shown to have McArdle s syndrome The patient had always had difficulty in performing sustained muscular exercise He fatigued early his muscles ached and if exertion continued would become stiff and swollen This sometimes was followed by myoglobinuria He had obtained a second wind repeatedly, and then was able to exercise for prolonged periods without difficulty The diagnosis of McArdle s syndrome was supported by lack of increase in blood level of lactate following ischemic exercise and was established by the lack of phosphorylase in his skeletal muscle Serum aldolase activity was slightly elevated The serum creatine phosphokinase (32) was 6 4 units at rest (normal value < 2 4 units) Immediately after 15 minutes leg exercise at 50 kgm per minute as described below the level increased to 9 1 units

Methods

General Procedure All studies were done in the morning after the patient had been fasting for 12—15 hours Teflon catheters were placed percutaneously with the Seldinger technique (3) in a brachial artery and in antecubital veins In one study a catheter was also introduced into the left femoral vein in the inguinal region and placed so that the tip was 12 cm below the inguinal ligament

Blood samples were taken in glass syringes placed in heparinized tubes chilled in ice and centrifuged at 3° C within 5 minutes Physiological saline without heparin was used to maintain patency of the catheters

The patient pedalled an electrically braked bicycle ergometer (17) Heart rate was recorded from electrocardiograms, and blood flow in the forearm was measured by venous occlusion plethysmography (2) In one study, flow was recorded with a Whitney strain gauge (35)

Materials Palmitic acid 1 C^{14} (New England Nuclear Corp) was converted to the sodium salt and mixed with approximately 2 moles human serum albumin per mole palmitate

The following drugs were used isoproterenol HCl (Isuprel, Winthrop) nicotinic acid 100 mg per ml (Invenex) and norepinephrine (Levophed, Winthrop)

Analytical Methods Ventilation was measured with a Tissot spirometer Consumption of oxygen production of carbon dioxide and ventilatory exchange ratio were calculated from analyses of expired air by the micro Scholander technique Samples of expired air were also taken from the Tissot spirometer for measurements of CO and $C^{14}O$ according to Fredrickson and Ono (12) Blood oxygen content and saturation were measured by the method of Van Slyke and Neill (34) Radioactivity in plasma FFA and the plasma concentration of FFA, *glycerol triglycerides* glucose and protein were measured as described previously (14 15) Lactate concentration was determined on blood plasma according to Loomis (20)

Calculations The calculations of the turnover rate and fractional oxidation

From the Cardiovascular Research Institute and the Department of Medicine University of California School of Medicine, San Francisco, California, U S A

The Second Wind Phenomenon in McArdle's Syndrome

By

Bengt B Pernow[1], Richard J Havel and Donald B Jennings[2]

Recent studies have clearly shown that the myopathy first described by McArdle (22) is the result of absence of skeletal muscle phosphorylase (23, 26, 27, 31, 33) Individuals with this deficiency cannot utilize their store of glycogen in skeletal muscle effectively Lactate, therefore, does not accumulate during exercise or hypoxia (22) Most (22, 27, 33) but not all (23) patients have an abnormally high concentration of glycogen in the muscle cells

The most common symptom in these subjects is an extremely low tolerance *to physical activity with unusual muscular* fatigue, pain and cramps during sustained muscular effort However, if the subject is able to sustain the exercise, these symptoms usually disappear This improved performance has been named "second wind" by Pearson et al (27) and has been observed by others (30 33) but its mechanism is not known Thomson et al (33) suggested direct degradation of glycogen to free glucose by amylo 1,4 glucosidase as an explanation However, such a mechanism should result in increased formation of lactate during exercise, this has never been observed in these patients Porte et al (30) recently discussed the possibility of mobilization of free fatty acids (FFA) as a more likely explanation They found that the tolerance to exercise was substantially increased by infusion of heparin or norepinephrine, both of which increase the arterial concentration of FFA

We tried to elucidate further the possible role of FFA in the development of second wind We found that the ability to develop a second wind was closely related to the prevailing level of FFA *in arterial blood plasma* *Increased* mobilization of FFA into the blood was not required, however We also showed that a second wind was obtained by procedures increasing blood flow to the muscles

Case report

Clinical features and demonstration of the second-wind phenomenon in our

[1] Present address Department of Clinical Physiology Serafimerlasarettet Karolinska Institutet Stockholm, Sweden

[2] Present address Department of Physiology Queen s University Kingston Ontario Canada

TABLE I Uptake and release of metabolites from the region drained by the femoral vein at rest and during exercise

	Free Fatty Acids (μmoles/ml)			Glycerol (μmoles/ml)		Lactate (μmoles/ml)		Glucose (mg/ml)	
Activity	Arterial	Uptake*	Release*	Arterial	A-V†	Arterial	A-V	Arterial	A-V
Rest	0.59	0.34	0.29	0.039	−0.016	0.51	−0.35	1.00	+0.06
200 kgm	0.81	0.13	0.08	0.120		0.42	+0.08	0.94	−0.02
250 kgm	0.94	0.09	0.06	0.110	−0.036	0.42	+0.03	0.85	0.00
300 kgm	1.01	0.13	0.08	0.107	−0.058	0.49	−0.07	0.82	+0.03

* *Based on fractional uptake of palmitate-1-C^{14} in relation to arterial and venous plasma levels of* FFA

† Arterio-venous difference

mmole/l (Table I). This amounts to 0.07 *mmoles of FFA per liter of whole* blood. Since one mole of FFA requires about 25 moles of oxygen for complete combustion and the mean arteriovenous difference of oxygen was 4.0 mmole/l, *complete oxidation of this FFA would* supply $25 \times 0.07/4.0 \times 100 = 44$ per cent of the oxidative metabolism of the tissues of the leg.

The fraction of infused C^{14} palmitate that appeared *in expired CO_2 during* exercise progressively increased to *78 per* cent. At all loads above 100 kgm/min about 25 per cent of expired CO_2 was *derived* from rapid oxidation *of circu*lating FFA.

The plasma concentration of glycerol increased considerably during *exercise*, the magnitude of rise being larger than *that of FFA*. The largest increase in glycerol was obtained *during the lightest* exercise; the plasma concentration was almost unchanged during the last 30 minutes of exercise. Glycerol was released from *tissues of* the leg both at rest and during exercise (Table I). The triglyceride level in blood varied little *during* exercise and *no measurable* arteriovenous difference of triglycerides was observed at rest or during exercise. Plasma concentration of glucose decreased from a resting value of 1.00 mg *per ml to 0.82 at the end of exercise* but uptake of glucose in leg tissues *could* not be demonstrated consistently. The *concentration of lactate in* arterial plasma was low at rest (0.5 mmole/l) and changed little during exercise. A *negative arteriovenous lactate difference* in the leg was observed at rest. During exercise venous lactate levels were lower *in two samples and higher in one*.

Oxygen content and saturation of arterial blood *were normal* at rest and *during* exercise. The *femoral* venous oxygen saturation at rest in the sitting position was 23 per cent, increased to *50 per cent at the start of* exercise and then was unchanged throughout the exercise (Fig. 2).

Effect of nicotinic acid on second stand (Fig. 1). After completing the first exercise *shown* in Fig. 1 the patient

of FFA and the percent of expired CO_2 derived from FFA during exercise have been described (15)

Results

STUDY A *Lipid metabolism during prolonged exercise (second wind)*

After the catheters were in position, C^{14} palmitate was infused through the venous catheter The patient rested for 40 minutes in a chair, and then began leg exercise at 50 kgm per minute (Fig 1) During the first few minutes, his heart rate rose rapidly and he complained of increasing fatigue and stiffness in the exercising muscles These symptoms, however, decreased rapidly after 7 minutes, and he could continue the exercise without further discomfort While his symptoms were lessening his heart rate decreased and stabilized at a lower rate The load was increased to 150 kgm per minute after 27 minutes of exercise and increased further 50 kgm per minute every 8 to 10 minutes up to 300 kgm per minute After a total period of 70 minutes, we asked the patient to stop although he said he was only mildly tired His ventilatory exchange ratio was 0 79 at rest and increased during exercise to about 0 90

Plasma concentration of FFA increased gradually during exercise but was stable during the last 10 minutes A further increase was observed in the sample taken 10 minutes after the end of exercise The turnover rate of FFA was doubled at the first load and was tripled at the end of exercise There was a small but consistant uptake of FFA in the leg both at rest and during exercise The amount of radioactivity in FFA (cpm ml) was 2 5 times greater at rest in arterial than in femoral venous blood while the specific activity (cpm/μmole) was about twice as high in arterial blood During exercise the arteriovenous difference of C^{14} palmitate decreased considerably The uptake of FFA in the leg calculated from these data if we assume that all FFA behaved like palmitate (14) decreased during exercise from a resting value of 0 34 mmole l to an average of 0 12

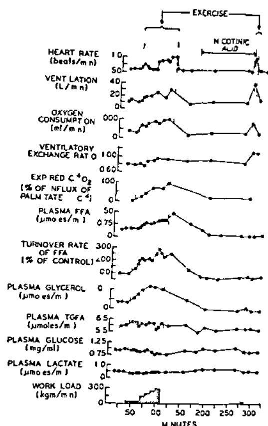
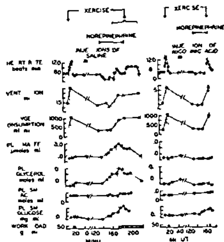
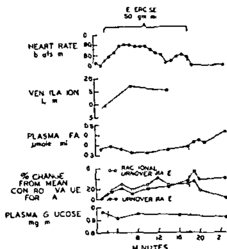


Fig 1 Data obtained during two consecutive exercise tests performed before and after intravenous infusion of nicotinic acid During the first test a second wind was obtained when the work load was increased gradually During the last 120 minutes of the resting period following the first exercise a continuous infusion of nicotinic acid was given immediately followed by the second exercise





F g 4 *Lef* Da a obta ned during wo exerc e sts w h a wo k load of 250 kgm per m n per f med b fo e and du ng nfus on of no ep neph ne During h es ng pe od be ween he wo es s sa ne as njec ed ntra enously as nd a ed by he a row *R gh* \ m ar ud ex ep ha n o n c ac d was gi en n ead of aline

F g 5 Da a ob a ned du ng a second w nd ex erc se of 16 m nu es dura on No e ha the u no e ra e of FFA as almost den cal af er 4 m nu es of exe cise when symp oms of fa gue and ffness we e p esent and heart ra e had n c eased o 90 and after 10 m nu es of exe c se when symp oms had subs ded and heart ra e was fa ng

n F g 4 Af er 2 l ours rest a constant nfu on of no ep nepl rine as tarted at a ra e of 20 μg per m nute During l e nfus on blood le els of FFA glycerol and glucose ncrea ed cons derably Heart ra e decreased Th rty m nutes la er tl e econd exerc e as started at 250 kgm m n He as able to con nue exercise for 50 m nutes and as then a b rantly stopped He was not fat gued and ated that he had no pa n or s ff ess n he legs Heart rate and ent la on ncreased less han dur ng the f rst e erc e The plasma concentrat on of FFA gl cerol and gluco e cont nued to nc ease a the beg nn ng of exerc se and hen decrea ed slo ly The infus on of no ep nepl rine was topped af er wh ch FFA and gl cerol le els fell rap dly Plasm a le els of lacta e rema ned he a ne as tl e res ng le els

Effect of n cot c ac d This study as repeated g ng ntravenous njections of 100 or 200 mg of n cot n c ac d instead of sal ne every 15 m nutes during the last 90 m nutes of the 2 1 2 hour resting period 6 Tl e total amount of n cot n c ac d g en as 1000 mgm As is een n F g 4 n cot n c ac d completely blocked he improved exerc se perform ance p oduced by norepinephrine This t me tl e pat ent could exerc se for only 1 n nute 20 seconds and then had to stop because l e as fat gued and the exerc s ng muscles ere stiff As n study A n co n c ac d reduced greatly the plasma concentration of FFA and glycerol Norep ephrine infus on d d not inc ease le els of FFA or gl cerol but tl e glucose le el ncreased to the same extent as n the control study when sal ne as g en

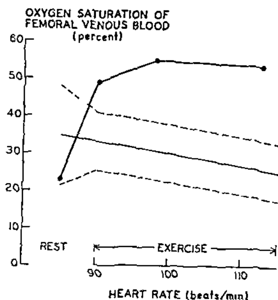


Fig 2 Oxygen saturation of femoral venous blood in relation to heart rate at rest (sitting on the bicycle) and during exercise Mean values ± 2 standard errors of the mean obtained in 18 healthy males of the same age group are shown at rest and during an identical type of exercise (27) (continuous and dashed lines)

rested in a comfortable chair for 2 1/2 hr During the last 90 minutes of this resting period, he received a constant intravenous infusion of nicotinic acid (6 5 mgm min) This was accompanied by decrease in FFA and glycerol levels in plasma, the latter to almost unmeasurable amounts (7) After a resting period of 170 minutes the patient resumed exercise at a load of 50 kgm/min He was completely exhausted after only 4 1 2 minutes, and the exercising muscles were tender and firm During this exercise there was a rapid and pronounced increase in heart rate, ventilation and oxygen consumption The increased ventilatory exchange ratio (to 1 23) suggested that he was hyperventilating The electrocardiogram was normal during and after the exercise No change was observed in FFA tri-

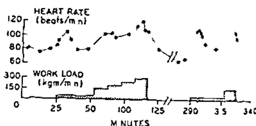


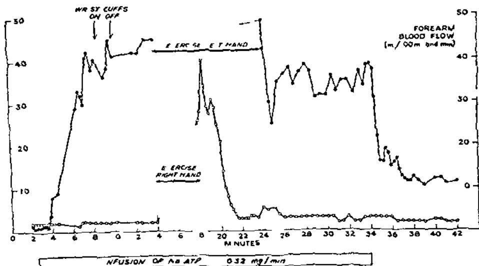
Fig 3 Two consecutive exercise tests showing that a second wind exercise could be reproduced in the same day when nicotinic acid was not given Note the rise and subsequent fall in heart rate when a given work load was sustained

glyceride, lactate, or glucose levels during or after the exercise The plasma *concentration of glycerol increased very* slightly

In a control study done on another day, the patient performed two exercise tests with a gradual increase in work load as described above and with a 2 1/2 hour interval between exercises (Fig 3) No blood samples were taken and no nicotinic acid was given A second wind was observed on both occasions The second exercise was arbitrarily stopped after 40 minutes without any fatigue or other symptoms

STUDY B *Improvement in physical working capacity by norepinephrine*

The improved exercise performance produced by norepinephrine (30) was confirmed (Fig 4) Before infusion of norepinephrine the patient was able to exercise at 250 kgm per minute for only 4 1 2 minutes His heart rate ventilation, and oxygen consumption increased plasma concentrations of FFA and glycerol were unaffected The patient then rested for 2 1/2 hours during which he was given intravenous injections of physiological saline solution as indicated



F g 8 Effect of infusing the sodium salt of adenos ne tr phosphate 0 43 mg ml 0 1ɔ m sod um chlor de solut on into the left brach al artery on ab l ty to perform hand exercise Room temperature was ma nta ned at 18 ? 19 7 to reduce flow to superf cial tissues Flow \ as measured ı h a \\ h tney stra n gauge \o wrist cuffs were appl ed except as nd cated Exerc se on the left was d continued arb trarily after 10 m nutes

and hε was able to continue indefinitely Essentially the same results were ob tained when this experiment was re peated on a separate day starting with the right hand

Forearm blood flow was measured before and after hand exercise with the rubber bulb When l e had become fa t gued after only 3 minutes ɔ0 seconds exerc se forearm blood flow l ad in creased 10 t mes above the resting level ı fell rapidly for 6 m nutes and then more slowly The work test was repeated ε eral times after d fferent intervals of rest The res ılts summarized in Fig 1 o s that a second wind could be ob tained if the exercise was repeated 3 and ɔ minutes after the end of the pre ceding exercise when the flow was still greatly increased When however the exercise was repeated after a 16 minute interval when the flow had stabilized near tl e resting level no second wind was obtained

Effect of isoproterenol On three occa sions performance of hand exercise was stud ed after the patient had been given 20 mg isoproterenol sublingually Heart rate increased gradually during the fol lowing 30 minutes to about 15 beats per minute above the resting level Forearm blood flow was almost doubled (one ob ervation Arterial blood pressure measured indirectly was not changed When hand exercise was performed 20 minutes after isoproterenol was given he invariably could exercise indefinitely (Fig 6B In a control study with a placebo tablet given *sublingually* the duration of an identical exercise was 2 minutes 30 seconds

Effect of nicotinic acid Fig 6C shows

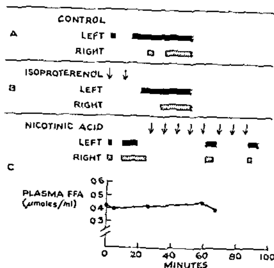


Fig 6 Duration of repeated exercises performed with the left and right hand A = without drugs, B = after sublingual administration of 10 mgm isoproterenol (indicated by arrows) given 20 and 10 minutes prior to the first left hand exercise In C each arrow indicates the injection of 2 mgm nicotinic acid into the right brachial artery The arterial concentration of FFA was measured in connection with the first three exercise tests in study C

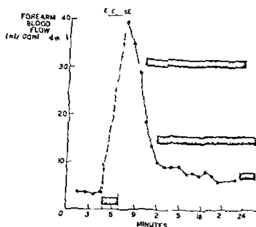


Fig 7 Ability to perform hand exercise in relation to forearm blood flow The figure summarizes the results of several studies and shows that a second wind was obtained only when the initial blood flow was more than about 3 times the resting level

These results, together with those obtained in study A, suggested that the spontaneous second wind in this patient was causally related to mobilization of FFA However, on another occasion a spontaneous second wind was unaccompanied by any change in the turnover rate of FFA Fig 5 shows the result of this study on which arterial concentration and turnover rate of FFA were determined every two minutes during exercise at a low load (50 kgm min) During the first 7 minutes, heart rate increased During this period, the patient experienced fatigue and stiffness in the anterior thighs these decreased abruptly and disappeared completely after 10 minutes The exercise was arbitrarily stopped after 16 minutes The blood level and turnover rate of FFA were almost identical at the time that the second wind occurred and during the first 4 minutes

STUDY C *Hand Exercise*

The patient squeezed a rubber bulb 60 times per minute He started the exercise with his left hand and was able to continue the rhythmic contractions for 2 minutes, 50 seconds (Fig 6A) He then had to stop because of a painful cramp in the forearm which persisted for several minutes After 10 minutes rest left hand exercise was resumed He could continue indefinitely but we asked him to stop after 35 minutes Eleven minutes after the second exercise with the left hand had started, when he had overcome the initial fatigue and felt able to go on indefinitely identical work was begun with the right hand After 2 minutes 40 seconds, he was unable to continue with the right hand the left hand was unaffected Five minutes later exercise of the right hand was resumed

Discussion

A comparison with earlier investigations in healthy young men (14, 15) shows that our patient could mobilize and utilize FFA as an energy source during leg exercise at least as well as normal subjects. The observed decrease in fractional uptake of FFA in the leg during exercise has also been observed in healthy young men (10).

The pronounced blocking effect of nicotinic acid on the release of FFA and glycerol from the adipose tissue (6, 7) has ben confirmed in this study. In contrast to what is seen in normal subjects (7) nicotinic acid markedly impaired our patient's physical working capacity: he was no longer able to do prolonged leg exercise at the lowest work load (50 kgm/min). After only 4 1/2 minutes he was exhausted and more than an hour passed before the stiffness in his legs disappeared. In addition the usual improvement in his performance produced by norepinephrine was completely blocked by nicotinic acid. Since the only known metabolic abnormality in patients with McArdle's syndrome is absence of skeletal muscle phosphorylase the difference in exercise performance after nicotinic acid between this patient and normal subjects confirms the importance of glycogenolysis to muscular function at the onset of exercise. This agrees with the observation (28) that a significant increase in lactate concentration in the blood draining working muscles occurs immediately following forearm exercise lasting only 5 seconds. The ability of normal subjects to exercise at moderate intensity when release of FFA has been completely blocked by nicotinic acid indicates that other substrates must be utilized efficiently. The higher ventilatory exchange ratio present under these conditions (6) suggests that more carbohydrate (blood glucose or muscle glycogen) is burned. It has recently been reported (25) that nicotinic acid increases the turnover rate of glucose in the dog. Exercising muscles are also able to oxidize stores of lipid in muscle cells (13). Evidently our patient was unable to utilize enough of these additional sources of energy to achieve a second wind when availablity of FFA was reduced by nicotinic acid.

It is unlikely that the effect of nicotinic acid on leg exercise performance resulted from changed peripheral circulation. The vasodilating action of nicotinic acid is well known and this effect lasts about 15 to 20 minutes after an injection (11). Repeated injections, however, produce a pronounced tachyphylaxis (6, 8) as reflected by the fact that the intensive flush which follows a single injection is no longer experienced on repeated injections. The circulatory effect after 8 injections during the course of 1 1/2 hours is therefore probably very small although it was not measured in this study.

The observations during leg exercise confirm and extend those of Porte et al (30) and demonstrate that the ability to *achieve a second wind is related to* the prevailing level of FFA in arterial blood plasma. This phenomenon appears to be analogous to that first reported by Pearson et al (27). In their patient exercise performance could be improved by intravenous administration of glucose,

the effect of intra arterial injection of nicotinic acid on ability to carry on hand exercise A second wind was obtained by repeated exercise in both arms, more completely in the right Arterial concentration of FFA did not change After 5 injections of 2 mg of nicotinic acid into the right brachial artery, the exercise was repeated with both hands The right hand was unable to perform more work than the left After 3 more injections, exercise performance of the right hand was greatly impaired, and he said he had more pain and fatigue in the forearm muscles than ever before The left hand worked twice as long as the right and was then fatigued but not painful

Effect of adenosine triphosphate The effects of prior exercise and of isoproterenol suggested that the basal rate of forearm blood flow could influence the patient's ability to perform hand exercise Accordingly, an experiment was devised in which blood flow to muscles of one forearm was increased by injection of the sodium salt of adenosine triphosphate (NaATP) into the brachial artery (10) The left side was selected for infusion because exercise performance on this side was generally poorer than on the right As shown in Fig 8 continuous intra arterial infusion of NaATP rapidly increased blood flow in the left forearm after about 1 minute to a level of about 40 ml per 100 ml forearm and minute after 4 minutes Inflation of wrist cuffs produced no detectable change indicating that the flow occurred primarily in the forearm and presumably in deep tissues The patient had no symptoms referable to the increased flow and no change in skin color was detected Blood flow on the right side was unchanged Finger temperature measured with a thermocouple, was 0 3° lower on the left side before the infusion and about 0 4° higher after 17 minutes of infusion After 11 1/2 minutes of infusion, exercise was begun in both hands with rubber bulbs After 3 minutes, 55 seconds, the patient was unable to continue exercise on the right side because of pain and cramping, but had no symptoms on the left Between 4 and 5 minutes of exercise he had moderate symptoms of fatigue on the left but had none thereafter Exercise on the left was arbitrarily stopped after 10 minutes Infusion of NaATP was stopped 10 minutes later Blood flow fell rapidly to about 12 ml per 100 ml and minute, but did not return to basal values for about 90 minutes Exercise was then repeated while physiological saline was infused into the left brachial artery Basal flow was 1 ml per 100 ml and minute on the right and 2 ml on the left Exercise could be continued for only 3 minutes 10 seconds in both hands but symptoms were more pronounced on the right Ten minutes later the exercise was repeated Basal blood flow was 3 ml per 100 ml and minute on the right and 5 ml on the left A second wind was obtained bilaterally The next day the test was repeated without arterial catheterization or measurement of blood flow The patient was able to exercise the left hand for only 2 minutes, 25 seconds Moderate symptoms occurred between 2 and 3 minutes on the right, but a second wind was obtained

and has suggested that the hyperemia of exercise is primarily the result of local release of potassium (18)

The amount of oxidizable substrates delivered to the muscles from the blood is a function of their arterial concentration and the rate of nutrient blood flow. Our studies allow us to conclude that increasing either of these variables can improve exercise performance in our patient. Since an increased concentration of FFA in arterial blood plasma was not required for the development of a spontaneous second wind during either leg or hand exercise, we conclude that mobilization of FFA from adipose tissue does not explain this phenomenon. Augmented or altered distribution of blood flow to the muscles could explain the second wind and support for such a mechanism has been adduced in the case of hand exercise

Our observations suggest that a catecholamine with specific effect on beta receptors would benefit patients with McArdle's syndrome. Isoproterenol increases arterial concentration of FFA (5, 24) as well as muscular blood flow (1, 9). We found that this drug effectively improved our patient's exercise performance [1]

Summary

A subject with myophosphorylase deficiency (McArdle's syndrome) in whom ability to perform muscular work was reproducibly improved following a short period of exercise to the point of fatigue and muscular discomfort (second wind phenomenon), has been studied. His ability to achieve a second wind during exercise on a bicycle ergometer was related directly to the level of FFA in arterial blood plasma. Performance was improved by increasing the rate of mobilization of free fatty acids into the blood with norepinephrine and was made worse by decreasing the rate of mobilization with nicotinic acid. Occurrence of a spontaneous second wind during exercise on the bicycle ergometer or during restricted exercise of forearm muscles produced by squeezing a rubber bulb was not necessarily accompanied by an increasing rate of fat mobilization from adipose tissue. Exercise to fatigue of forearm muscles was followed by a second wind that was confined to the exercised part and could be related to the rate of blood flow prevailing at the onset of exercise. Increasing forearm blood flow by infusion of adenosine triphosphate into a brachial artery improved exercise performance on the injected side. We conclude that augmented or altered distribution of blood flow to muscles could explain the second wind phenomenon in this patient but an increased rate of fat mobilization into the blood does not. His ability to exercise is, however, unusually dependent upon availability of free fatty acids to the working muscles

[1] The patient has found that taking 10 to 15 mg isoproterenol sublingually about 20 minutes before walking one fourth mile to his mailbox makes it possible for him to make the round trip with few or no symptoms in his legs.

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We thank Dr. Ellen Brown for advice and help in the studies of forearm blood flow and Dr. John T. Shepherd for suggesting the use of adenosine triphosphate to increase resting blood flow in forearm muscles

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fructose, lactate or fat emulsion They considered that a metabolic or circulatory readjustment could be responsible for the second wind It seems clear, however that an increased rate of mobilization of FFA is not required to produce a second wind in our patient, either during exercise involving large muscle groups (Fig 5) or following exercise of forearm muscles to the point of fatigue (Fig 6A) The latter exercise reproducibly produced a "local" second wind No increase in arterial concentration of FFA was produced by this type of exercise and confinement of the phenomenon to the previously exercised part eliminates the possibility that it is mediated by altered concentrations of blood born metabolites or hormones

Two observations support the possibility that circulatory changes may be responsible for the second wind observed during hand exercise First, improved performance was always obtained when the initial flow was three times above the normal resting level or greater (Fig 7) Second, increased blood flow produced by intra arterial injection of ATP substantially improved work performance of the injected muscles It is not possible to compare quantitatively the effects of ATP and prior exercise on forearm blood flow with the ability to perform hand exercise, since distribution of flow may have differed greatly in the two situations Much of the increased flow produced by ATP however must have involved deep tissues since exclusion of the hand had little effect on measured flow and eviden for increased skin flow was slight Previous exercise could also have produced metabolic changes within the muscles themselves which improved their ability to perform work Our observations do not require such an hypothesis, but they do not exclude the existence or importance of such changes The deleterious effect of infusing nicotinic acid into the brachial artery could have resulted either from blocking lipolysis of triglycerides that supply FFA directly to working muscles (15) or from redistribution of blood flow

Several observations seem to indicate that adjustment of the muscular blood flow during leg exercise is abnormal in our patient Oxygen saturation of the femoral venous blood (Fig 2) was significantly higher in relation to heart rate than in normal subjects of the same age group (29) The concentration of glycerol was considerably higher in femoral venous than in arterial blood both at rest and during exercise, which is in contrast to the virtually undetectable release of glycerol from the leg during exercise in most normal subjects (16) The distribution of the blood to the leg is probably abnormal with a higher percentage draining non muscular tissues such as adipose tissue (4) Possibly lactic acid normally released at the onset of exercise, facilitates local vasodilation Lundholm (21) found that infusion of lactic acid causes vasodilation in rabbits Alternatively failure of glycogenolysis might be accompanied by decreased release of potassium which occurs during muscular exercise (18 19) Recently Kjellmer has shown that potassium ion dilates resistance vessels in skeletal muscle in a manner closely resembling vasodilation during exercise

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Aus dem Radiobiologischen Institut, Karolinska Institutet, Stockholm, Schweden

Verschiebung der Sauerstoff-Hämoglobin-Dissoziationskurve der Maus nach Hypoxie entsprechend 6000 m Höhe

Von

BERNHARD TRIBUKAIT und REINER BRUMMER

Neben den Anpassungsprozessen eines Organismus an O_2-Mangelbedingungen wie gesteigerter Ventilation, Anstieg der Hämoglobinkonzentration und des Blutvolumens und erhöhter Kapillarisation mögen auch Änderungen der O_2Hb-Dissoziationskurve eine gewisse Bedeutung haben. Eine Linksverlagerung der Dissoziationskurve muss sich nämlich bei gegebenem Sauerstoffdruck auf die Sauerstoffaufnahme in der Lunge günstig, auf die Sauerstoffabgabe im Gewebe jedoch ungünstig auswirken und umgekehrt.

In der Literatur finden sich nur wenige und nicht einheitliche Angaben über das Verhalten der O_2Hb-Dissoziationskurve unter Hypoxie. Barcroft et al. (1922) haben bei einer Höhe von etwa 4300 m eine Linksverschiebung der Dissoziationskurve beschrieben. Demgegenüber haben Keys, Hall und Barron (1936) in 4—6000 m Höhe sowie Aste Salazar und Hurtado (1944) in 4500 m Höhe eine Rechtsverschiebung gefunden. Ebenfalls eine Rechtsverschiebung beim Kaninchen in 1800 m Höhe haben Wang, Wirz und Verzár (1951) auf eine kompensierte Acidose zurückgeführt. Beim neugeborenen Säuger (Mensch, Ziege, Schaf), deren Entwicklung bis zur Geburt unter O_2-Mangelbedingungen vor sich geht, ist die Dissoziationskurve linksverschoben (Bartels, Hilpert und Riegel, 1960). Gleiches gilt für verschiedene Höhentiere, u. a. ein Lama (Hall, Dill und Barron, 1936), wird aber auch bei dem dergleichen Familie zugehörigen und under O_2-Normalbedingungen lebenden Kamel gefunden (Bartels et al., 1963).

Die vorliegenden Untersuchungen gehen in eine Reihe von physiologischen und biochemischen Studien über die Ursachen der variirenden Strahlenempfindlichkeit von Mäusen im Anschluss an einen 10-tägigen Hypoxieaufenthalt entsprechend 6000 m Höhe ein. Unmittelbar nach einem derartigen Hypoxieaufenhalt ist die Strahlenempfindlichkeit mässig herabgesetzt, steigt während etwa 3—18 Stunden nach Hypoxie stark an, findum dann während etwa 24—72 Stunden nach Hypoxie in eine Phase herabgesetzter Strahlenempfindlichkeit über

zugehen, 96 Stunden nach Hypoxie beginnt sich die Strahlenempfindlichkeit zu normalisieren (Tribukait, 1966)

Die hier gestellte Frage ist, ob die Veranderungen der Strahlenempfindlichkeit mit eventuellen Sauerstoffdruckanderungen im Gewebe, im Zusammenhang stehen Die Sauerstoffkonzentration spielt namlich bei der Bestrahlungsreaktion von Zellen und Geweben eine bedeutende Rolle Bei einem Anstieg des Sauerstoffdrucks von 0 auf 20 mm Hg *ist mit einem Anstieg der Strahlenempfindlichkeit* um etwa das 2 ½- fache zu rechnen (Gray 1965)

Da die Hohe der Sauerstoffkonzentration im Gewebe neben den Bindungsverhaltnissen von Sauerstoff an Hamoglobin von der Gewebsdurchblutung und der mit dem Blut transportierten Sauerstoffmenge sowie dem Sauerstoffverbrauch der Zellen abhangt ist ohne weiteres klar dass hier nur ein kleiner Sektor einer komplexen Frage behandelt werden kann der sich zudem nur auf den Spezialfall eines 10-tagigen Hypoxieaufenthalts in 6000 m Hohe beschrankt Die gefundene starke und nach Abschluss der Hypoxie relativ langanhaltende Verschiebung der O_2Hb-Dissoziationskurven lassen eingehendere Untersuchungen bei unterschiedlichem Hypoxiegrad und verschiedener Hypoxiedauer wunschenwert erscheinen Auf die physiologische Bedeutung derartiger Verschiebungen fur die Sauerstoffversorgung der Gewebe mogen vergleichend physiologische Untersuchungen der Dissoziationskurven hinweisen Schmidt Nielsen *und* Larimer (1958) sowie Bartels (1964) fanden mit fallendem *Korpergewicht* bzw relativ steigendem Stoffwechsel eine zunehmende *Rechtsverlagerung der* Dissoziationskurven Gray und Steadman (1964) untersuchten die *Dissoziationskurven von* Maus, Ratte und Mensch und glaubten in deren unterschiedlicher Lage eine Erklarung dafur zu finden, dass Tumoren deren Zellen teilweise anoxisch sind, bei kleinen Tieren unter Sauerstoffatmung wahrend der Bestrahlung deutlich beeinflusst werden, ein vergleichbarer Effekt beim Menschen jedoch nicht vorzuliegen scheint.

Material und Methodik

Die *Untersuchungen* wurden an *mann*lichen zu Versuchsbeginn 23 g schweren NMRI Mausen durchgeführt Die Tiere erhielten Standardpellets und Wasser *ad libitum* Blutproben wurden von der coupierten Schwanzspitze entnommen, fur die Untersuchungen der O_2Hb-Dissoziationskurven und die Messungen des pH des Blutes unter Anwendung von heparinisierten Mikro-Hamatokrit Kapillaren

Die *O_2Hb Dissoziationskurven* des Blutes wurden nach dem von Niesel und Thews (1961) beschriebenen Prinzip bestimmt. Dabei wird ein Blutstropfen auf *einem Objekttrager in einer dunnen* Schicht aufgetragen und in einem abgeschlossenen *Gasraum in den* Messstrahl eines Photometers gebracht In *den* Messraum, der thermostatiert ist, werden Gase zum Oxygenieren bsw Desoxygenieren des Hamoglobins eingeleitet wobei gleichzeitig die Änderung der Absorption bei einer diskreten Wellenlange registriert wird

Bei den vorliegenden Versuchen wur-

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zugehen, 96 Stunden nach Hypoxie beginnt sich die Strahlenempfindlichkeit zu normalisieren (Tribukait, 1966)

Die hier gestellte Frage ist, ob die Veränderungen der Strahlenempfindlichkeit mit eventuellen Sauerstoffdruckänderungen im Gewebe im Zusammenhang stehen Die Sauerstoffkonzentration spielt namlich bei der Bestrahlungsreaktion von Zellen und Geweben eine bedeutende Rolle Bei einem Anstieg des Sauerstoffdrucks von 0 auf 20 mm Hg ist mit einem Anstieg der Strahlenempfindlichkeit um etwa das 2 ½- fache zu rechnen (Gray, 1965)

Da die Hohe der Sauerstoffkonzentration im Gewebe neben den Bindungsverhaltnissen von Sauerstoff an Hamoglobin von der Gewebsdurchblutung und der mit dem Blut transportierten Sauerstoffmenge sowie dem Sauerstoffverbrauch der Zellen abhangt ist ohne weiteres klar dass hier nur ein kleiner Sektor einer komplexen Frage behandelt werden kann der sich zudem nur auf den Spezialfall eines 10 tagigen Hypoxieaufenthalts in 6000 m Höhe beschrankt Die gefundene starke und nach Abschluss der Hypoxie relativ langanhaltende Verschiebung der O_2Hb-Dissoziationskurven lassen eingehendere Untersuchungen bei unterschiedlichem Hypoxiegrad und verschiedener Hypoxiedauer wunschenswert erscheinen Auf die physiologische Bedeutung derartiger Verschiebungen fur die Sauerstoffversorgung der Gewebe mogen vergleichend physiologische Untersuchungen der Dissoziationskurven hinweisen Schmidt Nielsen und Larimer (1958) sowie Bartels (1964) fanden mit fallendem Korpergewicht bzw relativ steigendem Stoffwechsel eine zunehmende Rechtsverlagerung der Dissoziationskurven Gray und Steadman (1964) untersuchten die Dissoziationskurven von Maus, Ratte und Mensch und glaubten in deren unterschiedlicher Lage eine Erklarung dafur zu finden, dass Tumoren deren Zellen teilweise anoxisch sind, bei kleinen Tieren unter Sauerstoffatmung während der Bestrahlung deutlich beeinflusst werden ein vergleichbarer Effekt beim Menschen jedoch nicht vorzuliegen scheint.

Material und Methodik

Die Untersuchungen wurden an männlichen, zu Versuchsbeginn 23 g schweren NMRI Mausen durchgefuhrt Die Tiere erhielten Standardpellets und Wasser *ad libitum* Blutproben wurden von der coupierten Schwanzspitze entnommen für die Untersuchungen der O_2Hb-Dissoziationskurven und die Messungen des pH des Blutes unter Anwendung von heparinisierten Mikro-Hamatokrit Kapillaren

Die *O_2Hb Dissoziationskurven* des Blutes wurden nach dem von Niesel und Thews (1961) beschriebenen Prinzip bestimmt Dabei wird ein Blutstropfen auf einem Objektrager in einer dunnen Schicht aufgetragen und in einem abgeschlossenen Gasraum in den Messstrahl eines Photometers gebracht In den Messraum der thermostatiert ist, werden Gase zum Oxygenieren bsw Desoxygenieren des Hamoglobins eingeleitet, wobei gleichzeitig die Anderung der Absorption bei einer diskreten Wellenlange registriert wird

Bei den vorliegenden Versuchen wur

Aus dem Radiobiologischen Institut, Karolinska Institutet, Stockholm, Schweden

Verschiebung der Sauerstoff-Hämoglobin-Dissoziationskurve der Maus nach Hypoxie entsprechend 6000 m Höhe

Von

Bernhard Triblkait und Reiner Brümmer

Neben den Anpassungsprozessen eines Organismus an O_2-Mangelbedingungen wie gesteigerter Ventilation, Anstieg der Hämoglobinkonzentration und des Blutvolumens und erhöhter Kapillarisation mögen auch Änderungen der O_2Hb-Dissoziationskurve eine gewisse Bedeutung haben. Eine Linksverlagerung der Dissoziationskurve muss sich nämlich bei gegebenem Sauerstoffdruck auf die Sauerstoffaufnahme in der Lunge günstig, auf die Sauerstoffabgabe im Gewebe jedoch ungünstig auswirken und umgekehrt.

In der Literatur finden sich nur wenige und nicht einheitliche Angaben über das Verhalten der O_2Hb-Dissoziationskurve unter Hypoxie. Barcroft et al. (1922) haben bei einer Höhe von etwa 4300 m eine Linksverschiebung der Dissoziationskurve beschrieben. Demgegenüber haben Keys, Hall und Barron (1936) in 4—6000 m Höhe sowie Aste-Salazar und Hurtado (1944) in 4500 m Höhe eine Rechtsverschiebung gefunden. Ebenfalls eine Rechtsverschiebung beim Kaninchen in 1800 m Höhe haben Wang, Wirz und Verzár (1951) auf eine kompensierte Acidose zurückgeführt. Beim neugeborenen Säuger (Mensch, Ziege, Schaf), deren Entwicklung bis zur Geburt unter O_2-Mangelbedingungen vor sich geht, ist die Dissoziationskurve linksverschoben (Bartels, Hilpert und Riegel, 1960). Gleiches gilt für verschiedene Höhentiere, u. a. ein Lama (Hall, Dill und Barron, 1936), wird aber auch bei dem dergleichen Familie zugehörigen und under O_2-Normalbedingungen lebenden Kamel gefunden (Bartels et al., 1963).

Die vorliegenden Untersuchungen gehen in eine Reihe von physiologischen und biochemischen Studien über die Ursachen der variirenden Strahlenempfindlichkeit von Mäusen im Anschluss an einen 10 tägigen Hypoxieaufenthalt entsprechend 6000 m Höhe ein. Unmittelbar nach einem derartigen Hypoxieaufenthalt ist die Strahlenempfindlichkeit mässig herabgesetzt, steigt während etwa 3—18 Stunden nach Hypoxie stark an, findum dann während etwa 24—72 Stunden nach Hypoxie in eine Phase herabgesetzter Strahlenempfindlichkeit über

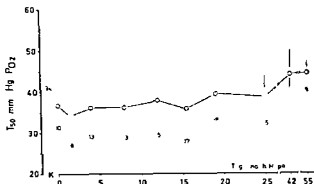


Abb 2 Sauerstoffdruck bei Halbsattigung der Dissoziationskurve (T_{50}) wahrend 55 Tage nach einem 10-tagigen Hypoxieaufenthalt entsprechend 6 000 m Hohe Mittelwerte mit ± Standardabweichung die Ziffern geben die Anzahl der Bestimmungen an K = Kontrollen

Durchstrommung automatisch reguliert waren hervorgerufen Zum Futtern der Tiere und Reinigen der Kafige befanden sich die Tiere taglich 5—10 Minuten in Normaldruck. Der Druckanstieg von 6000 m Hohe auf Meereshohe erfolgte innerhalb von 5 Minuten Die Umgebungsbedingungen fur die Kontrolltiere waren mit Ausahme der Druckverhaltnisse gleich

Ergebnisse

Der Einfluss von 10 tagiger Hypoxie auf die Lage der O Hb-Dissoziationskurven ausgedruckt durch den Sauerstoffdruck bei Halbsattigung ist Abb 2 zu entnehmen Da es wunschenswert erschien die zutage tretenden Veranderungen bis zur Normalisierung zu verfolgen die Kapazitat der Unterdruckkammer jedoch nicht ausreichte um gleichzeitig alle zu einem derartigen Versuch notwendigen Tiere Hypoxie auszusetzen befanden sich nur jeweils etwa 15 Tiere in Hypoxie Von diesen wurden Dissoziationskurven uber die Beobachtungszeit von 55 Tagen verteilt zusammen mit denen von entsprechenden Kontrolltieren aufgenommen Jeder der in Abb 2 wiedergegebenen Punkte reprasentiert somit Tiere die zu verschiedenen Zeitpunkten Hypoxie ausgesetzt worden waren Das Tiermaterial ist damit notwendigerweise weniger homogen denkbare zufallige und nicht fur das gesamte Tiermaterial gultige Veranderungen werden jedoch dadurch ausgeschlossen Eine Linksverschiebung der Dissoziationskurven ist ohne weiteres zu erkennen Die Halbsattigung sinkt von durchschnittlich 48 4 mm Hg auf einen Tiefstwert von 34 2 mm Hg am Tag 1—2 nach Hypoxieabschluss und erreicht erst nach etwa 4—6 Wochen wieder Normalwerte

Auch der fetale Hamoglobinanteil der ein Indikator fur eventuelle qualitative Abweichungen der Hamoglobinsynthese unter Hypoxie sein mag zeigt wahrend 4 Wochen nach Hypoxie noch erhohte Werte Abb 3) Die Schwankungen sind jedoch erheblich und der Anstieg von im Mittel etwa 1 1/2 % auf maximal 6 1/2 % zeigt dass under Hypoxie nur eine kleinere Quantitat der umschatzungsweise etwa 50 % erhohten Hamoglobinmenge als fetales Hamoglobin gebildet wird. Die bei der Streuung der Werte unsicher steigende Konzentration an fetalem Hamoglobin wahrend

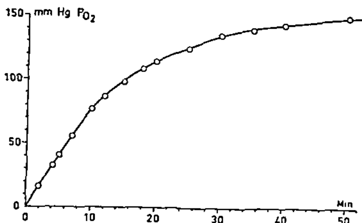


Abb. 1. Anstieg des Sauerstoffdrucks bei Einleiten von Luft in die mit Stickstoff gefüllte Messkammer

de ein Zeiss Spektrophotometer PMQ II mit einem TEW-Wandler, der das Messresultat von logarithmischer in eine lineare Skala überführt, verwendet. Als registrierendes Instrument diente ein Philips Linienschreiber. Ein so erhaltener Kurvenverlauf entspricht jedoch nur dann direkt einer O_2Hb-Dissoziationskurve, wenn der Anstieg des O_2-Drucks in der Messkammer zur Oxydation des Hämoglobins linear erfolgt. Um das zu prüfen, wurde mit einer pO_2-Elektrode (Beckman, Model 160) der O_2-Druckanstieg beim Einleiten von Luft in die mit Stickstoff gefüllte Messkammer verfolgt. Aus Abb. 1 geht hervor, dass der O_2-Druck zwischen O und 70 mm Hg mit der Zeit praktisch linear ansteigt.

Die Dissoziationskurven wurden bei einer Wellenlänge von 436 mμ und 37° C aufgenommen. Zum Desoxygenieren des Hämoglobins wurde Stickstoff (Sauerstoffgehalt 76 ppm), zum Oxygenieren ein Gasgemisch, bestehend aus 13,5 % O_2, 5,8 % CO_2 und 80,7 % N_2 verwendet. Zum Berechnen der Halbsättigungswerte, welche die O_2-Affinität des Blutes am einfachsten charakterisieren, wurden Eichgasgemische, bestehend aus 6,6 % O_2, 5,75 % CO_2 und 87,65 % N_2 sowie 3 % O_2, 6,4 % CO_2 und 90,6 % N_2 verwendet. Beim Oxygenieren bzw. Desoxygenieren des Blutes mit einem solchen Eichgasgemisch entspricht der Absorptionsverlauf zunächst einer Absorptionskurve, läuft dann aber in eine Gerade aus, deren Verlängerung die zuvor aufgenommene O_2Hb-Dissoziationskurve in einem Punkt schneidet, der der Sauerstoffsättigung des Blutes bei dem O_2-Partialdruck des Eichgasgemisches entspricht. Bei 19 Doppelbestimmungen betrug der Variationskoeffizient 11,6 % entsprechend 5,1 mm Hg (Mittelwert der Halbsättigung 43,8 mm Hg). Dabei wurde die zweite Blutprobe etwa 1 Stunde nach der ersten Bestimmung abgenommen.

Der *fetale Hämoglobinanteil* des Blutes wurde nach dem von Singer et al. (1951) angegebenen Alkali-Denaturierungsverfahren, *die Hämoglobinkonzentration* spektrophotometrisch als Oxyhämoglobin bei einer Wellenlänge von 545 mμ und das *pH des Blutes* bei 37° C mit Hilfe einer Beckman Mikroanordnung bestimmt.

Hypoxie wurde durch Unterdruck entsprechend 6000 m Höhe (= 354 mm Hg) in einer Kammer, deren Druck und

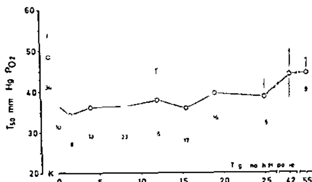


Abb 2 Sauerstoffdruck bei Halbsättigung der Dissoziationskurve (T_{50}) während 55 Tage nach einem 10-tägigen Hypoxieaufenthalt entsprechend 6 000 m Höhe Mittelwerte mit ± Standardabweichung die Ziffern geben die Anzahl der Bestimmungen an K = Kontrollen

Durchströmmung automatisch reguliert waren hervorgerufen Zum Füttern der Tiere und Reinigen der Käfige befanden sich die Tiere täglich 5—10 Minuten in Normaldruck. Der Druckanstieg von 6000 m Höhe auf Meereshöhe erfolgte innerhalb von 5 Minuten Die Umgebungsbedingungen für die Kontrolltiere waren mit Ausahme der Druckverhältnisse gleich

Ergebnisse

Der Einfluss von 10 tägiger Hypoxie auf die Lage der O Hb-Dissoziationskurven ausgedrückt durch den Sauerstoffdruck bei Halbsättigung ist Abb 2 zu entnehmen Da es wünschenswert erschien die zutage tretenden Veränderungen bis zur Normalisierung zu verfolgen die Kapazität der Unterdruckkammer jedoch nicht ausreichte um gleichzeitig alle zu einem derartigen Versuch notwendigen Tiere Hypoxie auszusetzen, befanden sich nur jeweils etwa 15 Tiere in Hypoxie Von diesen wurden Dissoziationskurven über die Beobachtungszeit von 55 Tagen verteilt zusammen mit denen von entsprechenden Kontrolltieren aufgenommen Jeder der in Abb 2 wiedergegebenen Punkte repräsentiert somit Tiere die zu verschiedenen Zeitpunkten Hypoxie ausgesetzt worden waren Das Tiermaterial ist damit notwendigerweise weniger homogen, denkbare zufällige und nicht für das gesamte Tiermaterial gültige Veränderungen werden jedoch dadurch ausgeschlossen Eine Linksverschiebung der Dissoziationskurven ist ohne weiteres zu erkennen Die Halbsättigung sinkt von durchschnittlich 48 4 mm Hg auf einen Tiefstwert von 34,2 mm Hg am Tag 1—2 nach Hypoxieabschluss und erreicht erst nach etwa 4—6 Wochen wieder Normalwerte

Auch der fetale Hämoglobinanteil der ein Indikator für eventuelle qualitative Abweichungen der Hämoglobinsynthese unter Hypoxie sein mag zeigt während 4 Wochen nach Hypoxie noch erhöhte Werte (Abb 3) Die Schwankungen sind jedoch erheblich und der Anstieg von im Mittel etwa 1 1/2 % auf maximal 6 1/2 % zeigt dass under Hypoxie nur eine kleinere Quantität der umschätzungsweise etwa 50 % erhöhten Hämoglobinmenge als fetales Hämoglobin gebildet wird Die bei der Streuung der Werte unsicher steigende Konzentration an fetalem Hämoglobin während

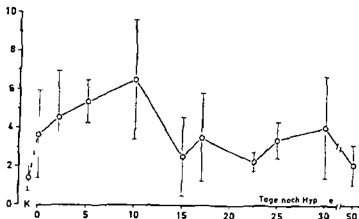


Abb 3 Fetale Hamoglobinkonzentration des Blutes wahrend 50 Tage nach einem 10 tagigen Hypoxieaufenthalt entsprechend 6000 m Hohe Mittelwerte mit Standardabweichung von je 10 Hypoxietieren und 13 Kontrolltieren (K)

der ersten 10 Tage nach Hypoxie entspricht dann den Erwartungen, wenn man damit rechnet, dass die vor Hypoxie gebildeten Erythrozyten entsprechend ihrer Lebenslange abgebaut werden und die unter Hypoxie gebildeten Erythrozyten mit einer hoheren Konzentration an fetalem Hamoglobin eine etwa normale Lebenslange haben Entsprechende Verhaltnisse fehlen jedoch bei der Verschiebung der Dissoziationskurve

Diese beiden Hypoxieveranderungen sind anhaltender als andere hier untersuchte Veranderungen Das pH des Vollblutes ist 24 Stunden nach Hypoxie wieder normal (Kontrollwert 7,33 ± 0,050, n = 19, unmittelbar nach Hypoxie 7,25 ± 0,018, n = 17, $p < 0{,}001$, 24 Stunden nach Hypoxie 7,31 ± 0,023, n = 8) Die Hamoglobinkonzentration ist nach 7 Tagen wieder normal (Kontrollwert 15,00 g% ± 1,209, n = 16, unmittelbar nach Hypoxie 21,38 g% ± 1,917, n = 9, 7 Tage nach Hypoxie 15,12 g% ± 0,957 n = 7)

In einer kleineren Versuchsreihe wurde ferner gepruft, ob 2 tagige Hypoxie entsprechend 6000 m Hohe bereits zu einer Verschiebung der Dissoziationskurve fuhrt Dabei ist mit Anderungen des pCO_2 des Blutes infolge Hyperventilation und des pH des Blutes sowie eines Anstiegs der Hamoglobinkonzentration infolge von Flussigkeitsverlusten zu rechnen, unter Hypoxie gebildetes Hamoglobin durfte jedoch noch nicht im peripheren Blut vorhanden sein (Tribukait 1963 a, b) Die Halbsattigung betrug 48 Stunden nach einem derartigen 2 tagigen Hypoxieaufenthalt 48,8 ± 4,78 mm Hg

Diskussion

Die vorliegenden Versuchsergebnisse zeigen eine deutlich Linksverschiebung der O_2Hb Dissoziationskurve nach Hypoxie Der Grad dieser Verschiebung ist so, dass eine funktionelle Bedeutung sowohl fur die O_2 Aufnahme in der Lunge als auch fur die O_2-Abgabe im Gewebe angenommen werden muss Am Beispiel zweier Kurvenverlaufe sei dieser Effekt einer Linksverschiebung besprochen In Abb 4 sind zwei zum selben Zeitpunkt von einem Kontrolltier und einem Tier 4 Tage nach einem 10 tagigen Hypoxieaufenthalt aufgenommene Kurven wie-

dergegeben Die Halbsattigungswerte betragen 45,4 bzw 27,7 mm Hg Aus einem Vergleich der beiden Kurven geht hervor, dass die Form der Dissoziationskurve die neben der durch die Halbsattigung charakterisierten Position in diesem Zusammenhang von Bedeutung ist, kaum grossere Veranderungen bei der Linksverlagerung erfahrt Bei normalem Sauerstoffdruck der Einatmungsluft mit Vollsattigung des arteriellen Blutes und einem angenommenen mittleren Sauerstoffdruck des venosen Blutes von 30 mm Hg wird das Blut zu 70 % (Normalkurve) bzw 45 % (linksverlagerte Kurve) ausgenutzt In 6000 m Hohe ausgehend von einem fur die Ratte errechneten alveolaren Sauerstoffdruck von 45 mm Hg (Tribukait 1963 a), betragt der Grad der Desaturierung etwa 25 % (Normalkurve) bzw 30 % (linksverlagerte Kurve) Je hoher der Stoffwechsel eines Gewebes und damit verbunden grosser die Sauerstoffausnutzung des Blutes ist desto bedeutungsvoller wird die relativ grossere noch zur Verfugung stehende Sauerstoffmenge bei Vorliegen eine linksverlagerten Dissoziationskurve unter Hypoxie Bei Berechnungen des Gewebssauerstoffdrucks vor allem kritischer Organe wie Herz und Gehirn unter anhaltender Hypoxie muss somit neben der Sauerstoffkapazitat des Blutes dem Grad der Kapillarisation der Durchblutung und dem Sauerstoffverbrauch auch die Lage der Dissoziationskurve berucksichtigt werden. Am kritischsten fur das Gewebe muss der Zeitraum zwischen einsetzender Hypoxie und dem kompensatorischen Anstieg der Sauerstoffkapazitat des Blutes und der damit verbundenen Linksverlagerung

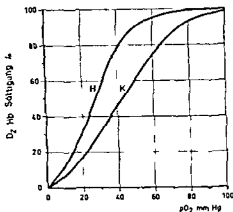


Abb 4 Dissoziationskurven eines Kontrolltiers (K) Halbsattigung = 45 4 mm Hg und einer Maus 4 Tage nach einem 10-tagigen Hypoxieaufenthalt (H) Halbsattigung = 27 7 mm Hg

der Dissoziationskurve sein Die initial einsetzende Hyperventilation führt zwar auch infolge des absinkenden CO_2-Drucks zu einer Linksverschiebung, der jedoch eine Acidose, mit der bei starkerer Hypoxie zu rechnen ist, entgegen wirkt

Wie eingangs besprochen worden ist sind mit einer Ausnahme (Barcroft et al 1922) rechtsverlagerte Dissoziationskurven beim Hohenaufenthalt beschrieben worden Welche Bedeutung der Grad des Sauerstoffmangels hat ob artspezifische Unterschiede vorliegen und in wie weit neuere Untersuchungsmethoden die alteren Befunden bestatigen konnen, mussen spatere Untersuchungen zeigen Der hier gefundene Mittelwert fur die Halbsattigung des normalen Blutes von 48 4 mm Hg stimmt mit dem von Schmidt Nielsen und Larimer (1958) angegebenen von 48 5 mm Hg uberein Gray und Steadman (1964) fanden bei einem pH von ~ 7 4 und einem PCO_2 von 40 mm Hg einen Halbsattigungswert von 41,5

mm Hg Abgesehen von Unterschieden der Untersuchungsmethoden und dem Behandlungsverfahren des Blutes durfte es eine „Standardkurve" der Maus nicht geben

Faktoren, die die Lage der Sauerstoffbindungskurve beeinflussen, haben Bartels und Riegel (1959), besonders im Hinblick auf die Veranderungen beim neugeborenen Sauger, besprochen Die Halbsattigung lasst sich danach zu Konzentrationsveranderungen des fetalen *Hamoglobinanteils*, aber auch zu morphologischen Anderungen der Erythrozyten wie denen des Diameters und der Oberflache korrelieren Weitere Ursachen der Lageveranderungen der Dissoziationskurve mogen in der Mikrostruktur und Biochemie der Zellen zu suchen sein Neben diesen strukturell fixierten Lageveranderungen, deren Dauer von der Erythrozytenlebenslange abhangt (Lebenslange des normalen Erythrozyten der NMRI-Maus etwa 46 Tage, Kunkel und Mauss 1966), wirken extrazellulare Faktoren wie CO_2 Druck und pH auf die Lage der Dissoziationskurve ein Die Dauer der gefundenen Linksverschiebung und die Tatsache, dass 2 tagige Hypoxie nicht zu einer Linksverschiebung fuhrt, machen es wahrscheinlich, dass strukturelle Veranderungen fur die Linksverschiebung verantwortlich sind Dabei ist der Anstieg des fetalen Hamoglobinanteils, fur den Werte gleicher Grossenordnung bei Untersuchungen mit der Gelelektrophorese gefunden wurden relativ so bescheiden dass dieser wenn uberhaupt in diesem Zusammenhang eine untergeordnete Bedeutung haben durfte Die durch Anderungen des CO_2 Drucks hervorgerufenen Verlagerungen der Dissoziationskurve sind hier nicht berucksichtigt worden Unmittelbar nach Hypoxie kann mit einem CO_2-Druck von rund 20 mm Hg gerechnet werden, der erst nach einer Reihe von Tagen langsam auf einen Normalwert ansteigt (Tribukait 1963 a) Entsprechend sind auch die Halbsattigungswerte wahrend der ersten Tage nach Hypoxie noch starker als aus den vorliegenden Daten hervor geht linksverschoben Wirkliche in vivo-Dissoziationskurven erhalt man aber erst dann, wenn der Sattigungs bzw Entsattigungsprozess auch von den in vivo entsprechenden CO_2 Druckveranderungen begleitet wird

Dass die Veranderungen der Strahlenempfindlichkeit nach Hypoxie mit der Linksverschiebung der Dissoziationskurve in direktem Zusammenhang stehen, erscheint bei der schlechten zeitlichen Uebereinstimmung der beiden Veranderungen und angesichts zahlreicher anderer physiologischer und biochemischer Veranderungen wenig wahrscheinlich Die vorliegenden Resultate mogen jedoch zum Verstandnis der Anpassung eines Organismus an Sauerstoffmangelbedingungen beitragen

Mit Unterstutzung durch den Staatlichen Rat fur Atomforschung sowie Cancerföreningen i Stockholm

Zusammenfassung

Die O_2Hb-Dissoziationskurve der Maus wurde mit einer spektrophotometrischen Mikromethode unter Standardbedingungen (37° C pCO₂ = 40 mm Hg) untersucht Dabei befindet sich ein Blutausstrich im Messstrahl eines Spektrophotometers in einer thermostatierteten Mess-

kammer, der Gase zum Oxygenieren und Desoxygenieren zugeführt werden Die dabei auftretenden Änderungen der Extinktion welche der O Hb-Dissoziationskurve entspechen, werden graphisch registriert Bei der normalen Maus mit einem pH des Blutes von 7,33 beträgt der Sauerstoffdruck für Halbsättigung durchschnittlich 48 4 mm Hg Nach einem 10 tägigen Hypoxieaufenthalt entsprechend 6000 m Höhe (= 354 mm Hg) ist die Dissoziationskurve linksverschoben und die Halbsättigung erreicht bei einem pH des Blutes von 7 31 1—2 Tage nach Hypoxieabschluss einen Tiefstwert von 34 2 mm Hg In vivo ist eine weitere Linksverschiebung infolge Absinken des arteriellen pCO auf etwa 20 mm Hg zu erwarten Normale Dissoziationskurven liegen erst nach 4—6 Wochen wieder vor Die Ursachen dieser Linksverschiebung und deren physiologische Bedeutung werden besprochen

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In the same sense of congratulation a number of Swedish colleagues have paid homage to Torgny Sjostrand on his birthday by sponsoring this volume Their names will be handed over to the celebrator in a special address on March 11 1967